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Research Communication

Dopaminergic Regulation of Glucose-induced Insulin Secretion through Dopamine D2 Receptors in the Pancreatic Islets *In Vitro*

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Summary

The stimulatory effect of dopamine through dopamine D2 receptor on glucose-induced insulin secretion was studied in the pancreatic islets *in vitro*. Dopamine significantly stimulated insulin secretion at a concentration of 10^{-8} M in the presence of high glucose (20 mM). The higher concentrations of dopamine (10^{-7}–10^{-4}) inhibited glucose-induced insulin secretion in the presence of both 4 mM and 20 mM glucose. Stimulatory and inhibitory effect of dopamine on glucose-induced insulin secretion was reverted by the addition of dopamine D2 receptor antagonists such as butaclamol and sulpiride. Norepinephrine (NE) at 10^{-4} M concentration inhibited the dopamine uptake as well as its stimulatory effect at 10^{-8} M concentration on glucose induced insulin secretion. Our results suggest that dopamine exerts a differential effect on glucose-induced insulin secretion through dopamine D2 receptor and it is essential for the regulation of glucose-induced insulin secretion by pancreatic islets.

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Keywords Dopamine; Norepinephrine; Dopamine D2 receptors; insulin secretion; diabetes; pancreatic islets; neurotransmitters.

INTRODUCTION

D-Glucose is the major physiological stimulus for insulin secretion. Phosphorylation of glucose to glucose-6-phosphate serves as the rate limiting step in glucose oxidation (*1*). Lesions in the pathways of glucose homeostasis lead to the pathogenesis of diabetes mellitus (*2*). Neurotransmitters are reported to have a key role in glucose-induced insulin secretion in the pancreatic islets and glucose homeostasis. Endogenously-synthesized islet catecholamines have been suggested to participate in paracrine regulation of insulin secretion. Epinephrine and norepinephrine has an antagonistic effect on insulin secretion and glucose uptake (*3, 4*). They also inhibit insulin-stimulated glycogenesis through inactivation of glycogen synthase and activation of phosphorylase with consequent accumulation of glucose-6-PO_4. At low concentration NE can bind and activate β-adrenergic receptors which in turn stimulate the insulin secretion from pancreatic islets and at high concentration they can bind to α_{2A} receptors and inhibit insulin secretion. Also, studies had shown that in diabetic condition α_{2A} receptors are more activated which brought out the insulin inhibition and in turn hyperglycemia (*5*). Rat islet cell membrane is equipped with α_{2A}-adrenoceptors (*6*) which are linked to adenylate cyclase inhibits insulin secretion. β_3 adrenoceptors stimulation also results in enhanced insulin secretion (*7*).

A link between the central nervous system and the pancreatic islets has been well established. The substantia nigra (SN) is one autonomic area in the central nervous system which plays an important role in controlling structure and activity of pancreatic islets. Lesions in the substantia nigra not only resulted in reduced size and number of islets cell populations but also decreased the content of insulin and glucagon in the pancreas (*8*). Studies have been focused on the existence of pathways between the SN and intermediolateral cells in the spinal cord and between the SN and hypothalamic paraventricular nucleus. The hypothalamic paraventricular nucleus has direct connections with the dorsal vagal complex (*9*). These reports underlined the role of SN in modulating the outflow of both sympathetic and parasympathetic signals that ultimately reach the pancreas. The central vagal connection with dopaminergic innervations is reported to reach the pancreatic islets through the parahypothalamic ventricular (PHV) nucleus while adrenergic and serotonergic innervations reach the pancreas through the brain stem (*8*).

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Secretory granules of pancreatic β cells have the ability to store substantial amounts of calcium, dopamine and serotonin (*10*). L-3, 4-dihydroxyphenylalanine (L-DOPA) is rapidly converted in islet β-cells to dopamine. In the pancreas of conscious rats dopamine D_1 receptors inhibited exocrine secretion mediated via sympathetic nerves. Dopamine accumulation in pancreatic islets is reported to have an inhibitory effect on glucose-stimulated insulin response (*11*). It is reported that increased hydrogen peroxide production increases MAO activity that augments the inhibitory effect of dopamine accumulation on insulin release (*11*). Dopamine is reported to suppress the stomatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through α-adrenergic mechanism and stimulates glucagon release through a β adrenergic mechanism. Sympathetic adrenergic α_1 and dopamine D_1 receptors are reported to be distributed on the β cells while β_2 receptors are located on the D cells and dopamine D2 receptors in the β neurons (*12*). Dysfunction of pancreatic islets play an important role in the etiology of diabetes as chronic hyperglycemia impairs islet function. It has been proposed that chronic hyperglycemia resulting from peripheral insulin resistance may impair secretogogue-induced insulin release. In the present study the effect of dopamine on glucose induced insulin secretion in the pancreatic islets *in vitro* was carried out. The results suggest that dopamine differentially regulate the pancreatic islets insulin secretion mediated through the dopamine D2 receptors.

EXPERIMENTAL PROCEDURES

[^{3}H] Dopamine Uptake Studies by Pancreatic Islets In Vitro

Pancreatic islets of male Wistar rats were aseptically dissected out into a sterile Petri-dish containing ice cold Hanks Balanced Salt Solution (HBSS) and isolated by standard collagenase digestion procedure (*13*). The islets were isolated in HEPES-buffered sodium free buffer (*14*) with the following composition: 137 mM Choline chloride, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM KH_2PO_4, 14.3 mM $KHCO_3$, 10 mM HEPES with 0.2% (w/v) BSA (Fraction V), equilibrated with 5% CO_2 and pH 7.3 at room temperature. Autoclaved triple distilled water was used in the preparation of the buffer. The pancreas was cut into small pieces and transferred to a sterile glass vial containing 2 ml collagenase type XI solution (1.5 mg/ml in HBSS), pH 7.4. The collagenase digestion was carried out for 20 min at 37 C in an environmental shaker with vigorous shaking (300 rpm/min). The tissue digest was filtered through 500 μm nylon screen and the filtrate was washed with three successive centrifugations and resuspended in cold HBSS medium. This filtrate was transferred to a sterile Petri-dish with a black base and examined under a dissection microscope. Islets visible as yellowish white spheres were handpicked carefully by finely drawn pasture pipettes and aseptically transferred to HBSS. The islets prepared by this method were used for all other experiments.

Then, 200 μl of islet suspension was transferred to tubes containing 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M concentrations of [^{3}H] DA. [^{3}H] DA concentrations were used along with 4 mM and 20 mM glucose. The final incubation volume was made up to 0.5 ml. The tubes were incubated for 1 h at 37 C in a shaking water bath.

The tubes were centrifuged after incubation at 1,500 *g* for 10 min at 4 C. The supernatant was aspirated out and pellet washed superficially with 0.2 ml of HBSS twice to remove free [^{3}H] DA. The pellet was digested with 100 μl of 1M KOH overnight and counted in a liquid scintillation counter with Cocktail-T to measure the [^{3}H] DA uptake.

Effect of Norepinephrine on Dopamine Uptake by Pancreatic Islets In Vitro

The islets prepared as per the above-mentioned procedure were transferred to tubes containing 10^{-8} M, 10^{-4} M concentrations of [^{3}H] DA and 10^{-8} M, 10^{-4} M concentrations of NE.

[^{3}H] DA concentrations were used along with 4 mM and 20 mM glucose. NE was used to study the [^{3}H] DA uptake along with glucose in this experiment. The final incubation volume was made up to 0.5 ml. The tubes were incubated for 2 hours at 37 C in a shaking water bath.

The tubes were centrifuged after incubation for 1 h at 1,500 *g* for 10 min at 4 C. The supernatant was aspirated out and pellet washed superficially with 0.2 ml of HBSS twice to remove free [^{3}H] DA or NE. The pellet was digested with 100 μl of 1M KOH overnight and counted in a liquid scintillation counter with Cocktail-T to measure the [^{3}H] DA uptake.

In Vitro *Insulin Secretion in the Presence of Different Concentrations of Dopamine, its Antagonist and Agonist*

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Similarly the islets were incubated with combinations of DA and its antagonists, dopamine agonists to study the effect of DA and its receptors on glucose-induced insulin secretion using radioimmunoassay.

Studies were also done in islets incubated for 1 h at 37 C with combinations of DA and NE at different concentrations to know the effect of NE on the role of DA in insulin secretion.

Secretory granules of pancreatic β cells have the ability to store substantial amounts of calcium, dopamine and serotonin (*10*). L-3, 4-dihydroxyphenylalanine (L-DOPA) is rapidly converted in islet β-cells to dopamine. In the pancreas of conscious rats dopamine D_1 receptors inhibited exocrine secretion mediated via sympathetic nerves. Dopamine accumulation in pancreatic islets is reported to have an inhibitory effect on glucose-stimulated insulin response (*11*). It is reported that increased hydrogen peroxide production increases MAO activity that augments the inhibitory effect of dopamine accumulation on insulin release (*11*). Dopamine is reported to suppress the stomatostatin secretion predominantly through activation of dopaminergic receptors, whereas it suppresses insulin release through α-adrenergic mechanism and stimulates glucagon release through a β adrenergic mechanism. Sympathetic adrenergic α_1 and dopamine D_1 receptors are reported to be distributed on the β cells while β_2 receptors are located on the D cells and dopamine D2 receptors in the β neurons (*12*). Dysfunction of pancreatic islets play an important role in the etiology of diabetes as chronic hyperglycemia impairs islet function. It has been proposed that chronic hyperglycemia resulting from peripheral insulin resistance may impair secretogogue-induced insulin release. In the present study the effect of dopamine on glucose induced insulin secretion in the pancreatic islets *in vitro* was carried out. The results suggest that dopamine differentially regulate the pancreatic islets insulin secretion mediated through the dopamine D2 receptors.

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RADIOIMMUNOASSAY OF INSULIN

The assay was done according to the procedure of BARC radioimmunoassay kit (BARC, Mumbai). The radioimmunoassay method is based on the competition of unlabelled insulin in the standard or samples and [^{125}I] insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody-polyethylene glycol (PEG) aided separation method. Measuring the radioactivity associated with bound fraction of sample and standards quantities insulin concentration of samples. A standard curve was plotted with $\%B/B_0$ on the Y-axis and insulin concentration/ml on the X-axis of a log-logit graph. $\%B/B_0$ was calculated as:

$$\frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of zero standard}} \times 100$$

Insulin concentration in the samples was determined from the standard curve plotted using MultiCalc^{TM} software (Wallac, Finland).

RESULTS

[^{3}H] Dopamine Uptake by Pancreatic Islets In Vitro

Our results showed pancreatic β cells can uptake dopamine at a concentration of 10^{-8} M in the presence of both 4 mM and 20 mM glucose. The presence of 4 mM and 20 mM glucose in the incubation medium can be considered equivalent to normal and diabetic states respectively. An increase in dopamine concentration up to 10^{-6} M decreased the dopamine uptake in both 4 mM and 20 mM glucose concentration. There was a concentration dependent differential uptake of [^{3}H] DA by pancreatic islets in the presence of both 4 mM and 20 mM glucose. The [^{3}H] DA uptake in the presence of 10^{-5} M was similar to 10^{-8} M [^{3}H] DA uptake in the presence of both 4 mM and 20 mM glucose. These results show that there is increased uptake of dopamine at 10^{-8} M and concentrations above 10^{-5} high concentrations of DA into the pancreatic islets in the presence of 4 mM and 20 mM glucose concentration (Fig. 1). [^{3}H] Dopamine uptake was inhibited by 10^{-4} M NE significantly ($P < 0.001$) while 10^{-8} M NE did not show any effect on glucose-induced insulin secretion. (Fig. 2).

Effect of Dopamine on Glucose-induced Insulin Secretion In Vitro

The pancreatic islets were incubated with 10^{-8} M, 10^{-7} M, 10^{-6} M 10^{-5} M, 10^{-4} M DA in the presence of 4 mM and 20 mM glucose. The presence of 4 mM and 20 mM glucose in the incubation medium is considered equivalent to normal and diabetic states respectively. Insulin secretion in the presence of 20 mM glucose was significantly higher ($P < 0.001$) than the secretion induced by 4 mM glucose. Addition of low concentration of dopamine (10^{-8} M) significantly ($P < 0.01$) enhanced the insulin secretion in the presence of 4 and 20 mM glucose when compared to cells incubated with 4 and 20 mM glucose alone (Fig. 3). As the concentration of DA increased to 10^{-7} M, 10^{-6} M 10^{-5} M and 10^{-4} the level of insulin secretion decreased significantly ($P < 0.001$) in the presence of both 4 mM and 20 mM glucose. Dopamine concentration 10^{-4} M produced the maximum inhibition of insulin secretion in the presence of 4 mM and 20 mM glucose incubated with the pancreatic islets (Fig. 3).

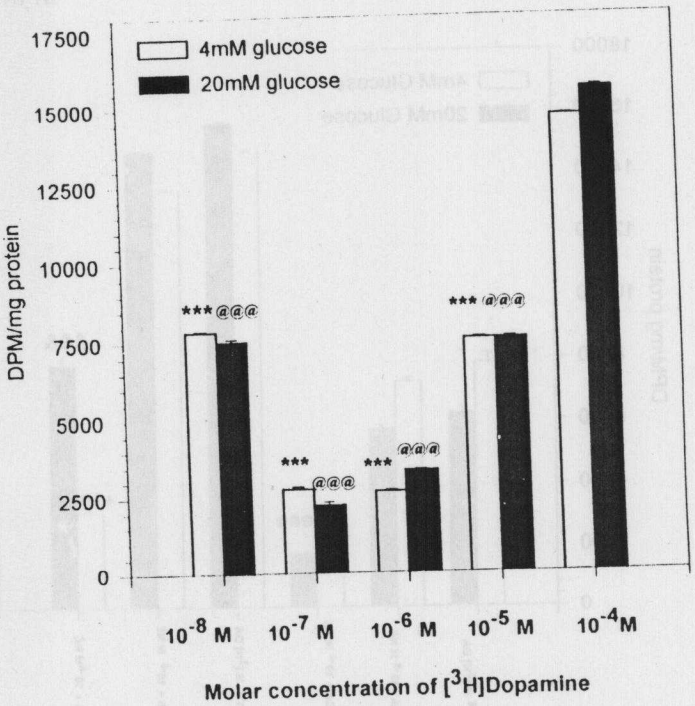

Figure 1. [^{3}H] Dopamine uptake in the pancreatic islets *in vitro*. ***$P < 0.001$ when compared with 10^{-4} M [^{3}H] dopamine + 4 mM glucose; @@@$P < 0.001$ when compared with 10^{-4} M [^{3}H] dopamine + 20 mM glucose. DA: Dopamine.

Effect of Dopamine Antagonists in Glucose-induced Insulin Secretion

Presence of dopamine antagonist in the culture medium along with dopamine showed that, butaclamol a general antagonist of dopamine, at 10^{-4} M concentration blocked the stimulatory effect of DA at 10^{-8} M concentration ($P < 0.001$) and also blocked the inhibitory effect of 10^{-4} M concentration of DA in the presence of 4 mM and 20 mM glucose (Fig. 4). Sulpiride, a potent dopamine D2 receptor antagonist, at 10^{-4} M concentration blocked ($P < 0.001$) the stimulatory effect of 10^{-8} M DA and inhibitory effect of 10^{-4} M concentration of DA in the presence of 4 mM and 20 mM glucose (Fig. 4). Thus the differential effect of dopamine on glucose-induced insulin secretion was mediated through its dopamine D2 receptors in the pancreatic islets.

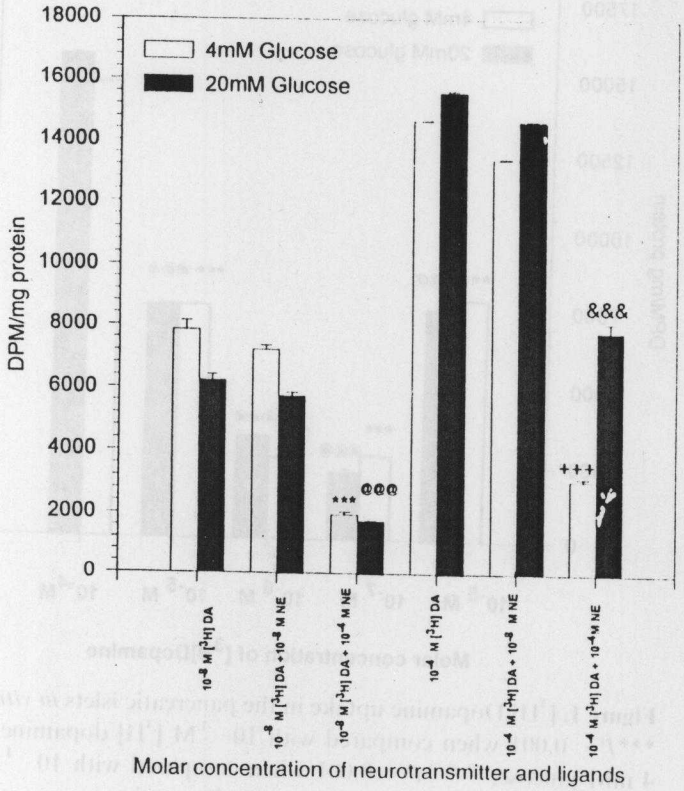

Figure 2. Effect of Norepinephrine on [^{3}H] Dopamine uptake in pancreatic islets *in vitro*. $^{***}P < 0.001$ when compared to 10^{-8} M [^{3}H] + 4 mM glucose; $^{@@@}P < 0.001$ when compared to 10^{-8} M [^{3}H] + 20 mM glucose; $^{+++}P < 0.001$ when compared to 10^{-4} M [^{3}H]DA + 4 mM glucose; $^{\&\&\&}P < 0.001$ when compared to 10^{-4} M [^{3}H]DA + 20 mM Glucose. DA: Dopamine; NE: Norepinephrine.

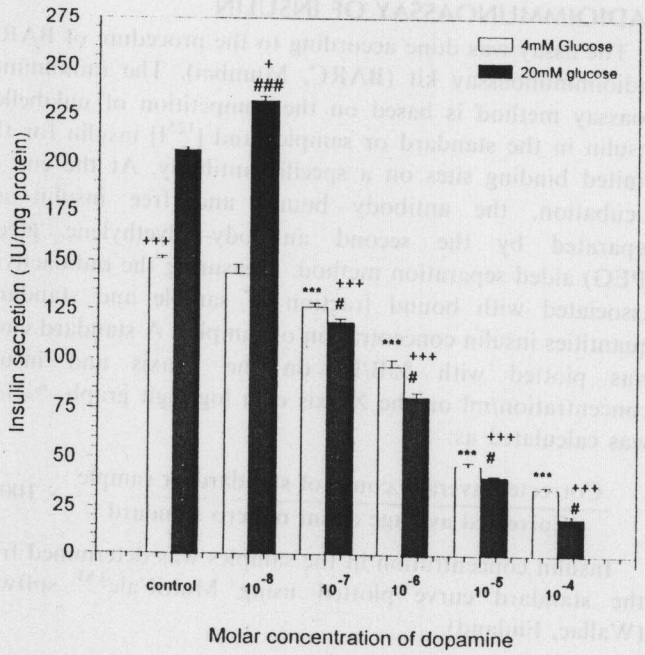

Figure 3. Effect of Dopamine (10^{-8} to 10^{-4} M) on glucose induced insulin secretion from pancreatic islets *in vitro*. $^{***}P < 0.001$ when compared to 4 mM glucose; $^{+++}P < 0.00$ when compared to 20 mM glucose; $^{+}P < 0.05$ when compared to 20 mM glucose; $^{\#}P < 0.05$ when compared to 4 M glucose + 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M DA; $^{\#\#\#}P < 0.001$ when compared to 4 mM glucose + 10^{-8} M DA. DA: Dopamine.

Effect of Norepinephrine on Dopaminergic Involvement in Glucose-induced Insulin Secretion

Norepinephrine in two concentrations (10^{-8} and 10^{-4} M) were added along with DA (10^{-8} M and 10^{-4} M) in 4 mM and 20 mM glucose and incubated with cells. It was observed that 10^{-8} M NE did not have any affect on the stimulatory effect of 10^{-8} M DA or on the inhibitory effect of 10^{-4} M DA in both the glucose concentrations incubated with cells. But 10^{-4} M NE in presence of 10^{-8} M DA in 4 mM and 20 mM glucose significantly inhibited ($P < 0.001$) insulin secretion induced by glucose overcoming the stimulatory effect of dopamine. A combination of 10^{-4} M NE and 10^{-4} M DA totally inhibited the glucose induced insulin secretion (Fig. 5).

DISCUSSION

Neurotransmitters especially catecholamines play an important role in insulin secretion. In the β cells, dopamine might be released from neurons innervating pancreatic islets and exocrine pancreas is an important source of dopamine (*28, 29*).

Dopamine is stored in the secretory granules of the pancreatic islets along with serotonin and calcium and could also be generated in pancreatic islets from its precursor L-dopa (*26, 30–32*). The uptake studies using [^{3}H] DA in the pancreatic islets revealed that the external concentration of dopamine itself has an effect on DA uptake. [^{3}H] DA uptake was found to be significantly higher at concentrations 10^{-4} M and 10^{-8} M compared to 10^{-7} M and 10^{-6} M DA in both 4 mM and 20 mM concentrations of glucose. Glucose-induced insulin secretion was found to be significantly increased in the presence of DA at a concentration 10^{-8} M, while 10^{-4} M DA completely inhibited the glucose induced insulin secretion. This is suggested to have an implication in insulin secretion as high concentrations of DA in the presence of glucose causes a reduction in insulin secretion. Dopamine is reported to modulate insulin secretion in the pancreatic islets (*15*). DA in the islets is essential for maintaining the equilibrium of insulin secretion. The function of islet β cells is controlled by a glucose sensor that operates at physiological glucose concentrations and acts in synergy with signals originating from hypothalamic neurons. Evidence exists that the extra pancreatic cells producing and secreting these neuro-endocrine signals also exhibit a glucose sensor activity and an ability to integrate nutrient and neuro-hormonal messages (*16*). Our studies in the pancreatic islets suggest that the DA exerts a

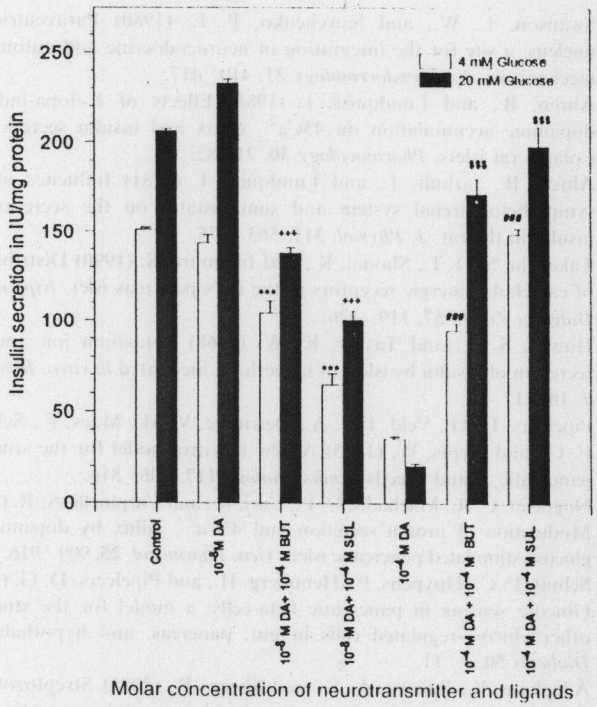

Figure 4. Effect of Dopamine Antagonists on glucose induced insulin secretion from pancreatic islets *in vitro*. $^{***}P < 0.001$ when compared with 4 mM glucose + 10^{-8} M DA; $^{+++}P < 0.001$ when compared with 20 mM glucose + 10^{-8} M DA; $^{\#\#\#}P < 0.001$ when compared with 4 mM glucose + 10^{-4} M DA; $^{\$\$\$}P < 0.001$ when compared with 20 mM glucose + 10^{-4} M DA. BUT: Butaclamol; SUL: Sulpiride; DA: Dopamine.

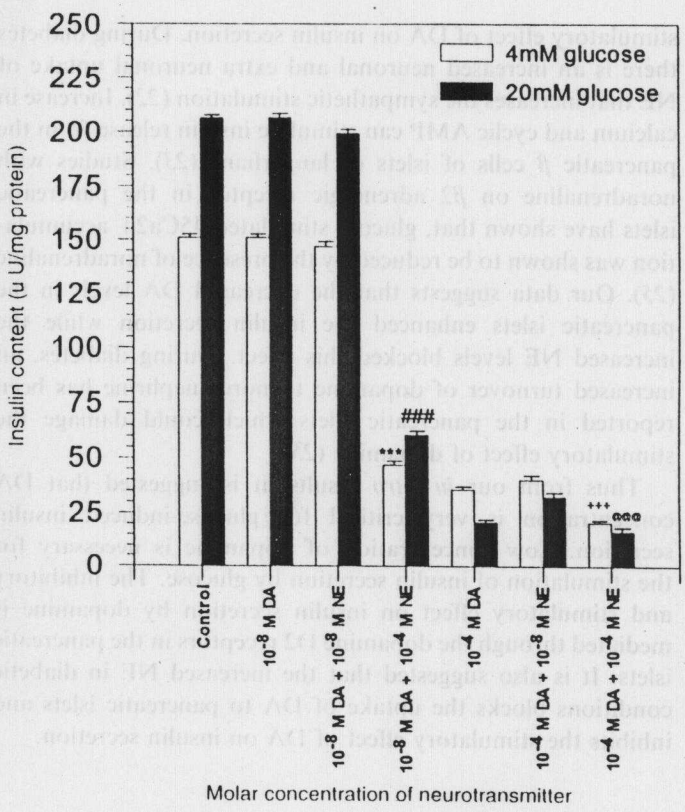

Figure 5. Effect of NE on the role DA in pancreatic islet glucose induced insulin secretion *in vitro*. Values are Mean S.E.M. of 4-6 separate determinations; $^{***}P < 0.001$ when compared to 4 mM glucose + 10^{-8} M DA; $^{\#\#\#}P < 0.001$ when compared to 20 mM glucose + 10^{-8} M DA; $^{+++}P < 0.001$ when compared to 4 mM glucose + 10^{-4} M DA; $^{@@@}P < 0.001$ when compared to 20 mM glucose + 10^{-4} M DA. NE: Norepinephrin; DA: Dopamine.

differential regulatory role in glucose-induced insulin secretion – its inhibitory effect is seen at increased concentrations and its stimulatory effect at lower concentrations.

To determine the modulation of insulin secretion by dopamine through its specific receptors, we used antagonists butaclamol and sulpiride. Butaclamol, a general antagonist of dopamine receptors blocked the stimulatory effect on glucose-induced insulin secretion at 10^{-8} M DA and inhibitory effect at 10^{-4} M DA. Presence of sulpiride, a potent dopamine D2 receptor antagonist to the pancreatic islets effectively blocked the dopaminergic action on insulin secretion. Identification of DA D2 receptors in the pancreatic islets of rodents suggests that these receptors would play an important role in insulin secretion (*26*). The results suggest that dopamine differentially regulates the pancreatic islets insulin secretion mediated through its DA D2 receptors. Our earlier studies reported that addition of forskolin, an activator of cAMP, antagonized the inhibitory effect of DA on insulin secretion (*19*).

NE at low concentration did not have any effect on the [^{3}H] DA uptake while at high concentration inhibited the uptake of DA at 10^{-8} M and 10^{-4} M in the presence of 4 mM and 20 mM glucose. Increased NE level is reported to inhibit the pancreatic islet function (*17*). The hypothalamic neuronal messages and the dopamine presence in the pancreatic islets cause the inhibition of insulin secretion during diabetes (*15*). Also, high concentrations of norepinephrine, dopamine, and serotonin in the pancreatic islets are reported to decrease glucose-stimulated insulin secretion (*18*). Thus, high concentration of NE blocked the uptake of DA into the pancreatic islets and this could affect the role of DA in glucose induced insulin secretion. Dopamine analogues like TL-99 and pergolide are reported to inhibit glucose-induced insulin secretion and increase glucose intolerance similar to epinephrine, mediated through the alpha-2-adrenergic receptors (*25, 27*). It has been previously reported that high concentrations of NE inhibited the glucose-induced insulin secretion (*18*). Norepinephrine is reported to have an inhibitory effect on insulin secretion in the pancreatic islets (*4*). Our results show that a high concentration of NE was inhibitory to DA uptake to pancreatic islets. Also, the stimulatory effect of DA on insulin secretion was inhibited. Low concentration of NE did not affect the DA uptake to pancreatic islets and the

stimulatory effect of DA on insulin secretion. During diabetes there is an increased neuronal and extra neuronal uptake of NE that increases the sympathetic stimulation (*22*). Increase in calcium and cyclic AMP can stimulate insulin release from the pancreatic β cells of islets of langerhans (*23*). Studies with noradrenaline on β2 adrenergic receptor in the pancreatic islets have shown that, glucose stimulated 45Ca2+ accumulation was shown to be reduced by the presence of noradrenaline (*25*). Our data suggests that the decreased DA levels in the pancreatic islets enhanced the insulin secretion while the increased NE levels blocked this effect. During diabetes, an increased turnover of dopamine to norepinephrine has been reported in the pancreatic islets which could damage the stimulatory effect of dopamine (*25*).

Thus from our *in vitro* results, it is suggested that DA concentration is very critical for glucose-induced insulin secretion. Low concentration of dopamine is necessary for the stimulation of insulin secretion by glucose. The inhibitory and stimulatory effect on insulin secretion by dopamine is mediated through the dopamine D2 receptors in the pancreatic islets. It is also suggested that the increased NE in diabetic conditions blocks the uptake of DA to pancreatic islets and inhibits the stimulatory effect of DA on insulin secretion.

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