Effects of adenosine A_1 receptor antagonism on insulin secretion

from rat pancreatic islets

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Summary

Adenosine is known to influence different kinds of cells, including β-cells of the pancreas. However, the role of this nucleoside in the regulation of insulin secretion is not fully elucidated. In the present study, the effects of adenosine A_1 receptor antagonism on insulin secretion from isolated rat pancreatic islets were tested using DPCPX, a selective adenosine A_1 receptor antagonist. It was demonstrated that pancreatic islets stimulated with 6.7 and 16.7 mM glucose and exposed to DPCPX released significantly more insulin compared with islets incubated with glucose alone. The insulin-secretory response to glucose and low forskolin appeared to be substantially potentiated by DPCPX, however, in the presence of glucose and high forskolin, DPCPX was ineffective. Moreover, DPCPX failed to change insulin secretion stimulated by the combination of glucose and dibutyryl-cAMP, a non-hydrolysable cAMP analogue. Studies on pancreatic islets also revealed that the potentiatory effect of DPCPX on glucose-induced insulin secretion was attenuated by H-89, a selective inhibitor of protein kinase A.

It was also demonstrated that formazan formation, reflecting metabolic activity of cells, was enhanced in islets exposed to DPCPX. Moreover, DPCPX was found to increase islet cAMP content, whereas ATP was not significantly changed.

These results indicate that adenosine A_1 receptor blockade in rat pancreatic islets potentiates insulin secretion induced by both physiological and supraphysiological glucose. This effect is proposed to be due to increased metabolic activity of cells and increased cAMP content.

Key words: insulin, secretion, adenosine, islets

Introduction

Under physiological conditions, insulin secretion is precisely regulated according to the actual demand of the organism. Glucose is the main physiological stimulator of insulin secretion. The insulinotropic action of glucose is preceded by a sequence of events involving its transport by facilitated diffusion and its oxidative metabolism, an increase in ATP/ADP ratio, closure of ATP-dependent potassium channels, membrane depolarisation, opening of voltage-dependent calcium channels and increase in cytosolic calcium. Finally, the rise in cytosolic calcium concentration triggers secretion of insulin. Moreover, additional signals in β-cells are generated to maintain the sustained secretory response to glucose (Henquin 2000, 2009).

The insulin-secretory response to glucose is known to be influenced by different stimulatory and inhibitory factors, such as dietary compounds (Newsholme *et al.* 2005, Nolan *et al.* 2006, Pinent *et al.* 2008), nervous system (Ahrén 1990) and incretins (Ahrén 2003, Baggio and Drucker 2007). Moreover, secretion of insulin undergoes paracrine regulation by glucagon and somatostatin (Schatz and Kullek 1980).

It is also known that the endocrine pancreas is influenced by purines (Petit *et al.* 2009). Effects induced by these compounds are mediated via purinergic receptors. Different types of purinergic receptors had been described in the pancreatic islet cells. Among them, adenosine A_1 receptor was identified indicating the potential regulatory role of adenosine in insulin release (Hillaire-Buys *et al.* 1987, 1994). Experimental data confirmed this assumption. It is known that in β-cells ATP is hydrolyzed to adenosine and this nucleoside is released from the cell, binds adenosine A_1 receptor, which is negatively coupled to adenylate cyclase, and insulin secretion is inhibited (Bertrand *et al.* 1989a, Hillaire-Buys *et al.* 1989). However, the physiological relevance of islet-derived adenosine in the regulation of β-cells is not fully elucidated. Studies on mice demonstrated that the magnitude of the second phase of insulin secretion was significantly greater in the case of pancreata obtained from adenosine A_1

receptor deficient animals compared with secretion observed in normal mice (Johansson *et al.* 2007). On the other hand, experiments on isolated pancreatic islets revealed that adenosine may exert an opposite effect on insulin secretion depending on nucleoside concentration. At high concentrations, adenosine was found to enhance insulin release, whereas at lower concentrations an inhibition was reported. The inhibition of hormone secretion is thought to result from the action of adenosine via adenosine A_1 receptor, whereas the opposite effect is proposed to be due to the intracellular metabolism of the nucleoside (Bertrand *et al.* 1989a, Petit *et al.* 2009). Data from the literature on the role of adenosine in the regulation of insulin secretion are not fully coherent since different effects were observed depending on nucleoside concentration, animal species and other experimental conditions (Ismail *et al.* 1977, Campbell and Taylor 1982, Bertrand *et al.* 1989a, Bertrand *et al.* 1989b, Petit *et al.* 1989, Verspohl *et al.* 2002, Töpfer *et al.* 2008, Hillaire-Buys *et al.* 1989). The aim of the present study was to determine the effects of adenosine A_1 receptor antagonism on insulin secretion from rat pancreatic islets.

Methods

Animals

Male Wistar rats weighing 280-350 g obtained from Brwinow (Poland) were used in all experiments. The rats were fed ad libitum a standard laboratory diet (Labofeed, Kcynia, Poland) and had free access to tap water. The animals were maintained in cages in an air-conditioned room with a 12:12-h dark-light cycle and a constant temperature 21 ±1 °C. The rats were killed by decapitation. The experiments were performed according to rules accepted by Local Ethical Commission for Investigation on Animals.

Islet isolation

Pancreatic islets were isolated by a collagenase digestion according to Lacy and Kostianovsky (1967). Hanks' solution (containing in mM: NaCl 137, KCl 5.36, $MgSO_4$ 0.81, Na_2HPO_4 0.34, KH_2PO_4 0.44, $CaCl_2$ 1.26, $NaHCO_3$ 4.17) gassed with 95% O_2/5% CO_2 was used during the isolation. The solution was injected into the common bile duct and the pancreas was excised. In each experiment, glands obtained from three rats were pooled, cut down with scissors and incubated with collagenase. Afterwards, islets were washed with Hanks' solution without the enzyme and were separated from the remaining exocrine tissue by hand picking under a stereomicroscope.

The effects of adenosine A_1 receptor blockade on insulin secretion

To study the effects of adenosine A_1 receptor blockade on insulin secretion, groups of 5 islets were incubated in 1 ml of Krebs-Ringer buffer (containing in mM: 115 NaCl, 24 $NaHCO_3$, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 0.5% bovine serum albumin). Before use the buffer was gassed with 95% O_2/5% CO_2 and its pH was adjusted to 7.4. In each experiment, islets were incubated for 90 min in a water bath at 37 °C in an atmosphere of O_2/CO_2 (95%/5%) with a gentle shaking. In the experiments, pancreatic islets were incubated in the presence of 2.8 mM glucose (basal secretion of insulin), 6.7 mM glucose (physiological concentration) or 16.7 mM glucose (supraphysiological concentration).

In the first part of our experiments, the effects of adenosine A_1 receptor blockade on insulin secretion induced by physiological and supraphysiological glucose were studied. For this purpose, pancreatic islets were stimulated with 6.7 or 16.7 mM glucose alone or glucose in the presence of 1, 10 and 50 μM DPCPX.

In the further part of the study, the effects of adenosine A_1 receptor blockade on insulin secretion induced by the combination of glucose and forskolin were tested. In these experiments, pancreatic islets were exposed to 6.7 mM glucose and 1 or 5 μM forskolin or glucose and forskolin in the presence of 10 and 50 μM DPCPX.

Moreover, the effects of adenosine A_1 receptor blockade on insulin secretion induced by the combination of glucose and dibutyryl-cAMP were studied. Isolated pancreatic islets were incubated in the buffer containing 6.7 mM glucose and 0.5 mM dibutyryl-cAMP or glucose and dibutyryl-cAMP in the presence of 1, 10 and 50 μM DPCPX.

Additionally, the effect of H-89, a specific inhibitor of protein kinase A, on glucose-induced insulin secretion in the presence of DPCPX was tested. In these experiments, islets were incubated in the Krebs-Ringer buffer containing 6.7 mM glucose alone, glucose and 20 μM H-89, glucose and 50 μM DPCPX or glucose, H-89 and DPCPX.

Stock solutions of DPCPX, forskolin and H-89 were prepared in dimethyl sulfoxide. The final concentration of this solvent in the buffer with islets was less that 0.1%.

Measurement of insulin concentration

At the end of islet incubations, aliquots of the incubation medium were sampled and stored (-20 °C) for insulin determination. Insulin concentrations were measured radioimmunologically using kits specific for rat hormone.

The effects of adenosine A_1 receptor blockade on islet ATP content and formazan formation

To determine the effect of A_1 receptor blockade on ATP content, pancreatic islets were incubated for 60 min at 37 °C in Krebs-Ringer buffer containing 6.7 mM glucose alone or in the combination with 50 μM DPCPX. After the incubation, trichloroacetic acid was added, ATP was isolated (Gembal *et al.* 1993, Szkudelski 2007) and its concentration was measured

by a luminometric method with a kit containing luciferase and luciferin. In order to determine the effect of adenosine A_1 receptor blockade on formazan formation from MTT, pancreatic islets were incubated for 90 min at 37 °C in Krebs-Ringer buffer containing 0.5 mg/ml MTT and 6.7 mM glucose or MTT, glucose and 50 μM DPCPX. Then, islets were incubated with isopropanol at room temperature for an additional 90 min (Szkudelski 2007). Finally, the absorbance of isopropanol was read at 560 nm (Janjic and Wollheim 1992).

The effects of adenosine A_1 receptor blockade on islet cAMP concentrations

In this part of the study, pancreatic islets were incubated for 30 min in the buffer containing 6.7 mM glucose alone, glucose and 5 μM forskolin or glucose and 50 μM DPCPX. At the end of the incubation, cAMP was isolated, according to the method used for isolation of ATP (Szkudelski, 2007), and total cAMP was measured by a non-acetylation EIA procedure according to the instruction enclosed by the manufacturer.

Reagents. D-glucose, forskolin, dibutyryl-cAMP, bovine serum albumin (fatty acid free), DPCPX (8-cyclopentyl-1,3-dipropylxanthine), thiazolyl blue tetrazolium bromide (MTT) and all reagents used to measure ATP, to prepare Krebs-Ringer buffer and Hanks' solution were obtained from Sigma (St. Louis, USA). Collagenase P was from Roche Diagnostics GmbH (Mannheim, Germany), DMSO (dimethyl sulfoxide) and H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide) were from ICN Biomedicals, Inc. (Ohio, USA). cAMP kits (EIA) were provided by Amersham (Buckinghamshire, UK) and insulin kits were from Millipore Corporation (USA).

Statistical analysis

The means ± S.E.M. from 3 independent experiments in quadruplicates were evaluated statistically using analysis of variance and Duncan's multiple range test. Differences were considered significant at P<0.05.

Results

The effects of adenosine A_1 receptor blockade on insulin secretion

The basal secretion of insulin, measured at 2.8 mM glucose, was 0.85 ng/islet/90 min, whereas stimulation of pancreatic islets with 6.7 and 16.7 mM glucose increased secretion of insulin to 3.75 and 14.01 ng/islet/90 min, respectively (Fig. 1). These results demonstrate that pancreatic islets used in our studies were metabolically active and effectively responded to physiological and supraphysiological glucose.

It was demonstrated that the insulin-secretory response to glucose was significantly changed by adenosine A_1 receptor blockade. Insulin secretion induced by 6.7 mM glucose appeared to be increased in the presence of 1, 10 and 50 μM DPCPX. Similar effect was noticed in the case of high glucose. Insulin secretion induced by 16.7 mM glucose was markedly potentiated by islet exposure to 1, 10 and 50 μM DPCPX. However, the effects induced by DPCPX were not concentration-dependent (Fig. 1).

In the further part of the study, the effect of adenosine A_1 receptor blockade on insulin secretion induced by the combination of 6.7 mM glucose and 1 or 5 μM forskolin was tested. It was found that insulin secretion induced by glucose and 1 μM forskolin was substantially potentiated in the presence of 10 and 50 μM DPCPX. However, when insulin secretion was induced by glucose and 5 μM forskolin, DPCPX appeared to be ineffective and hormone secretion was similar in the case of islets incubated with or without DPCPX (Fig. 2).

Insulin secretion induced by the combination of 6.7 mM glucose and 0.5 mM dibutyryl-cAMP was significantly increased compared with secretion found in the case of 6.7 mM glucose alone. However, the insulin-secretory response of β-cells to 6.7 mM glucose and 0.5 mM dibutyryl-cAMP was unchanged by 1, 10 or 50 μM DPCPX (Fig. 3).

It was also demonstrated that 20 μM H-89, a direct inhibitor of protein kinase A, did not change insulin secretion induced by 6.7 mM glucose. However, H-89 decreased insulin secretion from islets exposed to 6.7 mM glucose and 50 μM DPCPX (Fig. 4).

The effects of adenosine A_1 receptor blockade on islet ATP content and formazan formation

Experiments on isolated pancreatic islets demonstrated that adenosine A_1 receptor blockade did not significantly change ATP content. In these experiments, ATP content was similar in the case of islets incubated with 6.7 mM glucose alone and islets incubated with glucose and 50 μM DPCPX (Fig. 5). Formazan formation from MTT appeared to be significantly higher in islets incubated with 6.7 mM glucose and 50 μM DPCPX compared with islets exposed to glucose alone (Fig. 5).

The effects of adenosine A_1 receptor blockade on islet cAMP concentrations

As expected, incubations of isolated pancreatic islets for 30 min in the presence of 6.7 mM glucose and 5 μM forskolin substantially increased cAMP compared with results obtained in islets stimulated with glucose alone (data not shown). It was also demonstrated that 50 μM DPCPX significantly augmented cAMP content in pancreatic islets compared with cAMP in islets incubated without DPCPX (Fig. 5).

Discussion

In the present study, freshly isolated rat pancreatic islets were used to elucidate the role of endogenous adenosine in the regulation of insulin secretion. In the majority of previous investigations, insulin-secreting cells were exposed to exogenous adenosine or to its non-hydrolysable analogues (Campbell and Taylor 1982, Bertrand *et al.* 1989b, Petit *et al.* 1989). Under these conditions, it is difficult to clearly distinguish the effects evoked by endogenous and exogenous nucleoside. In the present experiments, a selective adenosine A_1 receptor antagonist, DPCPX, was used to suppress the influence of islet-derived adenosine.

It was demonstrated that pancreatic islets stimulated with glucose and exposed to DPCPX released significantly more insulin compared with secretion observed in the presence of glucose alone. The potentiatory effect of DPCPX was found at both physiological and supraphysiological glucose concentrations. This indicates that adenosine A_1 receptor blockade enhances glucose-induced insulin secretion at a broad range of stimulatory glucose concentrations.

Data from literature indicate that in the majority of experiments adenosine was found to inhibit insulin secretion, however, some discrepancies may be found depending on experimental conditions. In studies on INS-1 cells, exogenous adenosine inhibited glucose-induced insulin secretion and this effect was suppressed by DPCPX (Verspohl *et al.* 2002). In experiments on rat pancreatic islets, adenosine was also demonstrated to decrease insulin secretion. In rat islets, the inhibitory effect of 10 µM adenosine on insulin secretion induced by 4-16 mM glucose was observed (Ismail *et al.* 1977). Similarly, adenosine (1 µM - 2.5 mM) and its analogue (L-PIA; 1 nM-10 µM) reduced insulin secretion from perifused rat pancreatic islets exposed to 15 mM glucose (Bertrand *et al.* 1989b). The inhibitory action of adenosine (1-100 µM), PIA (0.1-10 µM) and 2-deoxyadenosine (10 mM) on insulin secretion was also shown when rat islets were stimulated with 20 mM glucose (Campbell and Taylor 1982). A

small, transient inhibitory effect of 50 µM adenosine on insulin secretion stimulated with 15 mM glucose was demonstrated in mouse islets, however, 500 µM adenosine potentiated insulin release (Bertrand *et al.* 1989a).

The rise in glucose-stimulated insulin secretion observed in our studies in the presence of DPCPX may result from increased metabolic activity of β-cells as demonstrated in experiments with MTT. In islets exposed to glucose and DPCPX, reduction of MTT to formazan was significantly greater compared with islets incubated with glucose alone. It is known that in the insulin secreting cells, the rate of formazan production from MTT correlates with glucose oxidation and utilization (Janjic and Wollheim 1992).

Our study also demonstrated increased cAMP content in islets incubated with DPCPX. Since adenosine A_1 receptor is negatively coupled to adenylate cyclase (Hillaire-Buys *et al.* 1989), suppression of adenosine action by DPCPX is supposed to increase the activity of adenylate cyclise leading to the rise in islet cAMP concentrations. It is known that the increase in cAMP in β-cells significantly enhances the insulin secretory response to glucose (Yamada *et al.* 2002). Therefore, the increased cAMP may significantly contribute to the rise in insulin secretion in the presence of DPCPX. This assumption was confirmed by experimental data. It was demonstrated that the potentiatory effect of DPCPX on glucose-induced insulin secretion was completely suppressed in the presence of dibutyryl-cAMP, a non-hydrolysable cAMP analogue. Under these conditions, the intracellular concentration of cAMP (in the form of dibutyryl-cAMP) was very high and the effect of DPCPX on insulin secretion did not reveal.

Additional evidence supporting the important role of cAMP in the action of DPCPX was obtained in experiments with H-89, a specific inhibitor of protein kinase A. In these experiments, the potentiatory effect of DPCPX on glucose-induced insulin secretion appeared to be attenuated by H-89. This indicates that the rise in cAMP and a concomitant activation of

protein kinase A are of major importance for the potentiatory effect of DPCPX on glucose-induced insulin secretion.

Interestingly, our study revealed that DPCPX substantially enhanced insulin secretion stimulated by the combination of glucose and forskolin. However, this effect was found only in the presence of 1 µM forskolin, whereas in the presence of 5 µM forskolin, similarly to results obtained in experiments with dibutyryl-cAMP, DPCPX appeared to be ineffective. The increased secretion of insulin in the presence of DPCPX and low forskolin indicates that blockade of adenosine A_1 receptor substantially enhances secretion of insulin probably as a result of the synergistic action of forskolin and DPCPX on cAMP. In the presence of 5 µM forskolin, the effect of DPCPX is negligible because high forskolin itself generates substantial increase in cAMP in islet cells.

In conclusion, it was demonstrated that adenosine A_1 receptor blockade in rat pancreatic islets significantly enhances insulin secretion induced by physiological and supraphysiological glucose. The potentiatory effect of adenosine A_1 receptor blockade on insulin secretion seems to result from increased metabolic activity of islet cells and from a concomitant increase in cAMP content. The obtained results confirm previous data that islet-derived adenosine attenuates insulin secretion. Moreover, these outcomes point at the possibility of the potential use of adenosine A_1 receptor antagonists as compounds enhancing insulin secretion.

Figure legend

Fig. 1. The effects of DPCPX on glucose-induced insulin secretion from rat pancreatic islets. Islets were incubated in the buffer containing 6.7 (top, gray bars) or 16.7 (bottom, black bars) mM glucose without DPCPX or with DPCPX. Open bars – incubations with 2.8 mM glucose. Values represent means ±SEM of 12 determinations from 3 separate experiments.

* - differences statistically significant vs. incubations without DPCPX ($p < 0.05$)

Fig. 2. The effects of DPCPX on insulin secretion stimulated by glucose and forskolin. Islets were incubated in the buffer containing 6.7 mM glucose alone (open bar), glucose and 1 (gray bars) or 5 (black bars) µM forskolin without DPCPX or in the presence of DPCPX. Values represent means ±SEM of 12 determinations from 3 separate experiments.

* - differences statistically significant vs. incubations without DPCPX ($p < 0.05$)

Fig. 3. The effects of DPCPX on insulin secretion stimulated by glucose and dibutyryl-cAMP. Islets were incubated in the buffer containing 6.7 mM glucose alone (open bar) or glucose and 0.5 mM dibutyryl-cAMP (black bars) without DPCPX or in the presence of DPCPX. Values represent means ±SEM of 12 determinations from 3 separate experiments.

Fig. 4. The effects of H-89 on insulin secretion stimulated by glucose and DPCPX. Islets were incubated in the buffer containing 6.7 mM glucose alone (open bar), glucose and 50 µM DPCPX, glucose and 20 µM H-89 or glucose, DPCPX and H-89 (black bars). Values represent means ±SEM of 12 determinations from 3 separate experiments.

* - differences statistically significant vs. incubations with DPCPX alone ($p < 0.05$)

Fig. 5. The effects of DPCPX on formazan formation (open bars), cAMP (gray bars) and ATP content (black bars) in rat pancreatic islets. To study the effects of DPCPX on formazan formation, islets were incubated in the presence of 0.5 mg/ml MTT for 90 min. To determine the effects of DPCPX on cAMP and ATP content, pancreatic islets were incubated for 30 min. In each case, 6.7 mM glucose was present in the incubation medium. Values represent means ±SEM of 12 determinations from 3 separate experiments.

* - differences statistically significant vs. incubations without DPCPX ($p < 0.05$)

References

AHRÉN B, KARLSSON S, LINDSKOG S: Cholinergic regulation of the endocrine pancreas. *Prog Brain Res* **84**: 209-218, 1990.

AHRÉN B: Gut peptides and type 2 diabetes mellitus treatment. *Curr Diab Rep* **3**: 365-372, 2003.

BAGGIO LL, DRUCKER DJ**:** Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132**: 2131-2157, 2007.

BERTRAND G, NENQUIN M, HENQUIN JC, Comparison of the inhibition of insulin release by activation of adenosine and alpha 2-adrenergic receptors in rat beta-cells. *Biochem J* **259**: 223-228, 1989b.

BERTRAND G, PETIT P, BOZEM M, HENQUIN JC: Membrane and intracellular effects of adenosine in mouse pancreatic beta-cells. *Am J Physiol* **257**: 473-478, 1989a.

CAMPBELL IL, TAYLOR KW: Effects of adenosine, 2-deoxyadenosine and N6-phenylisopropyladenosine on rat islet function and metabolism. *Biochem J* **204**: 689-696, 1982.

GEMBAL M, DETIMARY P, GILON P, GAO ZY, HENQUIN JC: Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K+ channels in mouse B cells. *J Clin Invest* **91**: 871-880, 1993.

HENQUIN JC: Regulation of insulin secretion: a matter of phase control and amplitude modulation. *Diabetologia* **52**: 739-751, 2009.

HENQUIN JC: Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* **49**: 1751-1760, 2000.

HILLAIRE-BUYS D, CHAPAL J, BERTRAND G, PETIT P, LOUBATIÈRES-MARIANI MM: Purinergic receptors on insulin-secreting cells. *Fundam Clin Pharmacol* **8**: 117-127, 1994.

HILLAIRE-BUYS D, BERTRAND G, GROSS R, LOUBATIÈRES-MARIANI MM: Evidence for an inhibitory A1 subtype adenosine receptor on pancreatic insulin-secreting cells. *Eur J Pharmacol* **136**: 109-112, 1987.

HILLAIRE-BUYS D, GROSS R, LOUBATIÈRES-MARIANI MM, RIBES G: Effect of pertussis toxin on A_1-receptor-mediated inhibition of insulin secretion. *Br J Pharmacol* **96**: 3-4, 1989.

ISMAIL NA, EL DENSHARY ESM, MONTAGUE W : Adenosine and the regulation of insulin secretion by isolated rat islets of Langerhans. *Biochem J* **164**, 409-413, 1977.

JANJIC D, WOLLHEIM CB: Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. *Diabetologia* **35**: 482-485, 1992.

JOHANSSON SM, SALEHI A, SANDSTRÖM ME, WESTERBLAD H, LUNDQUIST I, CARLSSON PO, FREDHOLM BB, KATZ A: A1 receptor deficiency causes increased insulin and glucagon secretion in mice. *Biochem Pharmacol* **74**: 1628-1635, 2007.

LACY PE, KOSTIANOVSKY M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **16**: 35-39, 1967.

NEWSHOLME P, BRENNAN L, RUBI B, MAECHLER P: New insights into amino acid metabolism, beta-cell function and diabetes. *Clin Sci (Lond.)* **108**: 185-94, 2005.

NOLAN CJ, MADIRAJU MS, DELGHINGARO-AUGUSTO V, PEYOT ML, PRENTKI M: Fatty acid signaling in the beta-cell and insulin secretion. *Diabetes* **55**: 16-23, 2006.

PETIT P, LAJOIX AD, GROSS R: P2 purinergic signalling in the pancreatic beta-cell: control of insulin secretion and pharmacology. *Eur J Pharm Sci* **37**: 67-75, 2009.

PETIT P, BERTRAND G, SCHMEER W, HENQUIN JC: Effects of extracellular adenine nucleotides on the electrical, ionic and secretory events in mouse pancreatic beta-cells. *Br J Pharmacol* **98**: 875-882, 1989.

PINENT M, CASTELL A, BAIGES I, MONTAGUT G, AROLA L, ARDÉVOL A: bioactivity of flavonoids on insulin-secreting cells. *Comprehensive Reviews in Food Science and Food Safety* **7**: 299–308, 2008.

SCHATZ H, KULLEK U: Studies on the local (paracrine) actions of glucagon, somatostatin and insulin in isolated islets of rat pancreas. *FEBS Lett* **122**: 207-210, 1980.

SZKUDELSKI T: Resveratrol-induced inhibition of insulin secretion from rat pancreatic islets: evidence for pivotal role of metabolic disturbances. *Am J Physiol Endocrinol Metab* **293**: 901-907, 2007.

TÖPFER M, BURBIEL CE, MÜLLER CE, KNITTEL J, VERSPOHL EJ: Modulation of insulin release by adenosine A1 receptor agonists and antagonists in INS-1 cells: the possible contribution of 86Rb+ efflux and 45Ca2+ uptake. *Cell Biochem Funct* **26**: 833-843, 2008.

VERSPOHL EJ, JOHANNWILLE B, WAHEED A, NEYE H: Effect of purinergic agonists and antagonists on insulin secretion from INS-1 cells (insulinoma cell line) and rat pancreatic islets. *Can J Physiol Pharmacol* **80**: 562-568, 2002.

YAMADA S, KOMATSU M, SATO Y, YAMAUCHI K, KOJIMA I, AIZAWA T, HASHIZUME K: Time-dependent stimulation of insulin exocytosis by 3',5'-cyclic adenosine monophosphate in the rat islet beta-cell. *Endocrinology* **143**: 4203-4209, 2002.
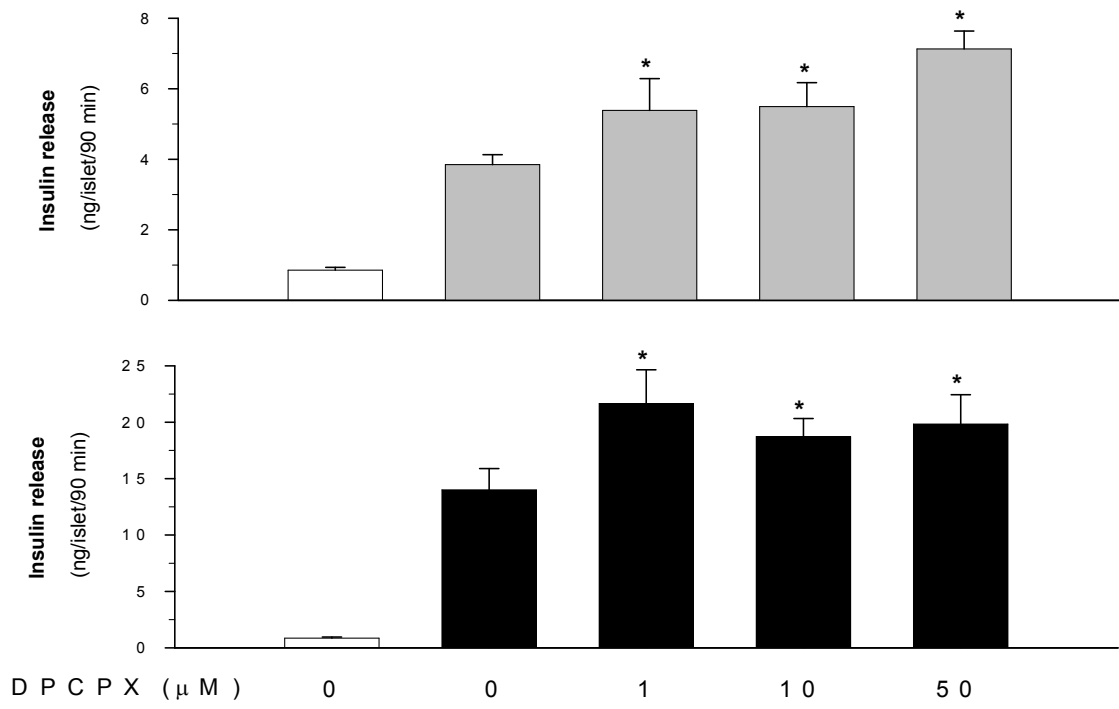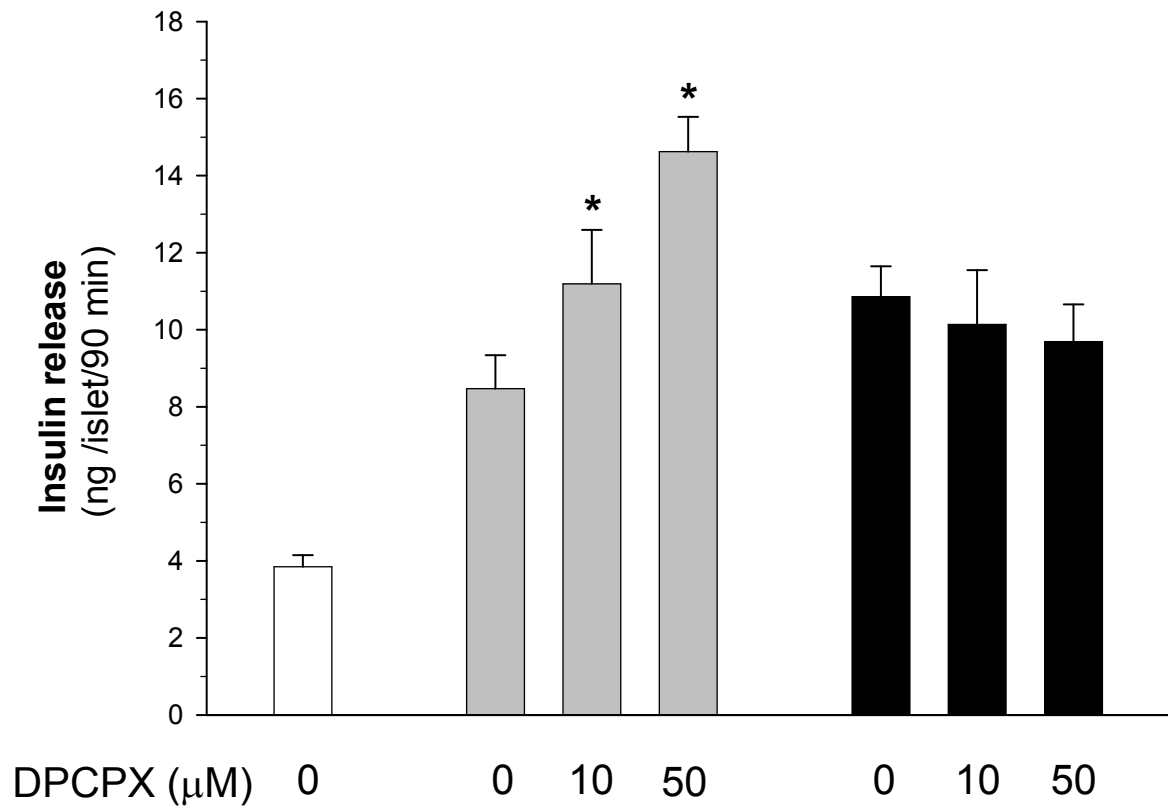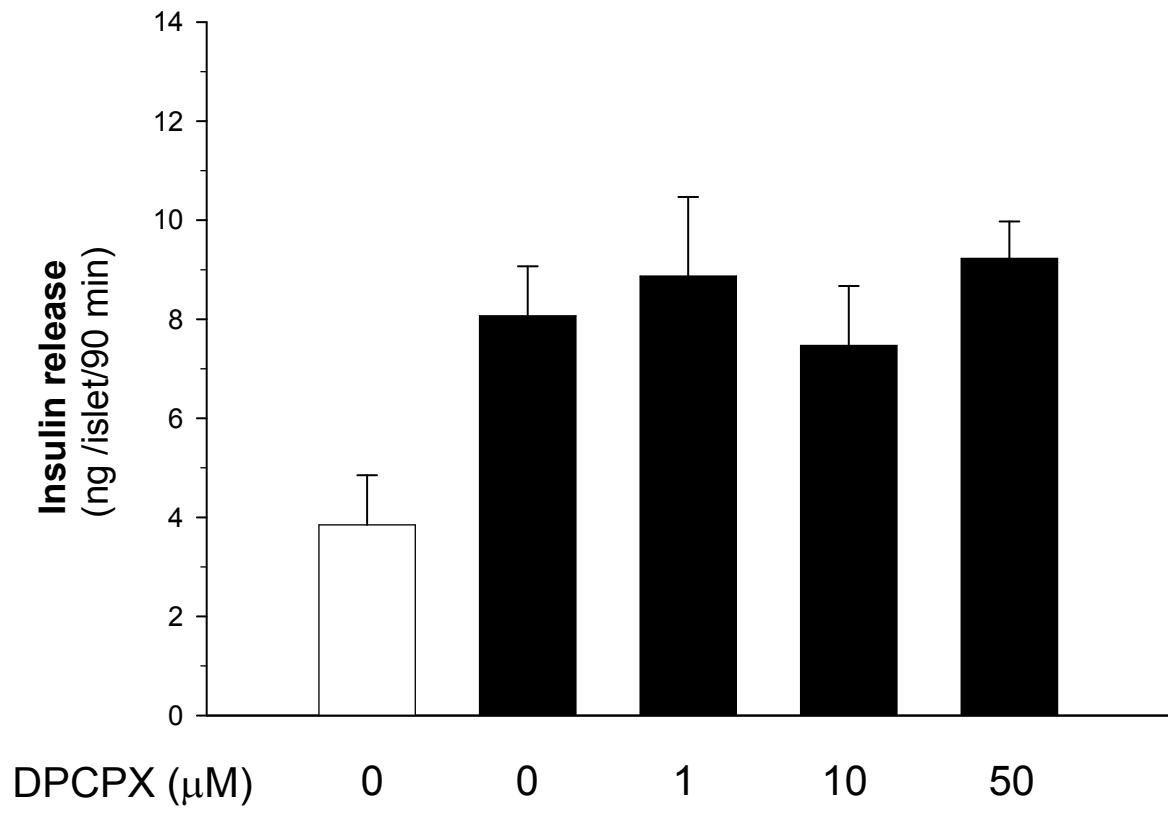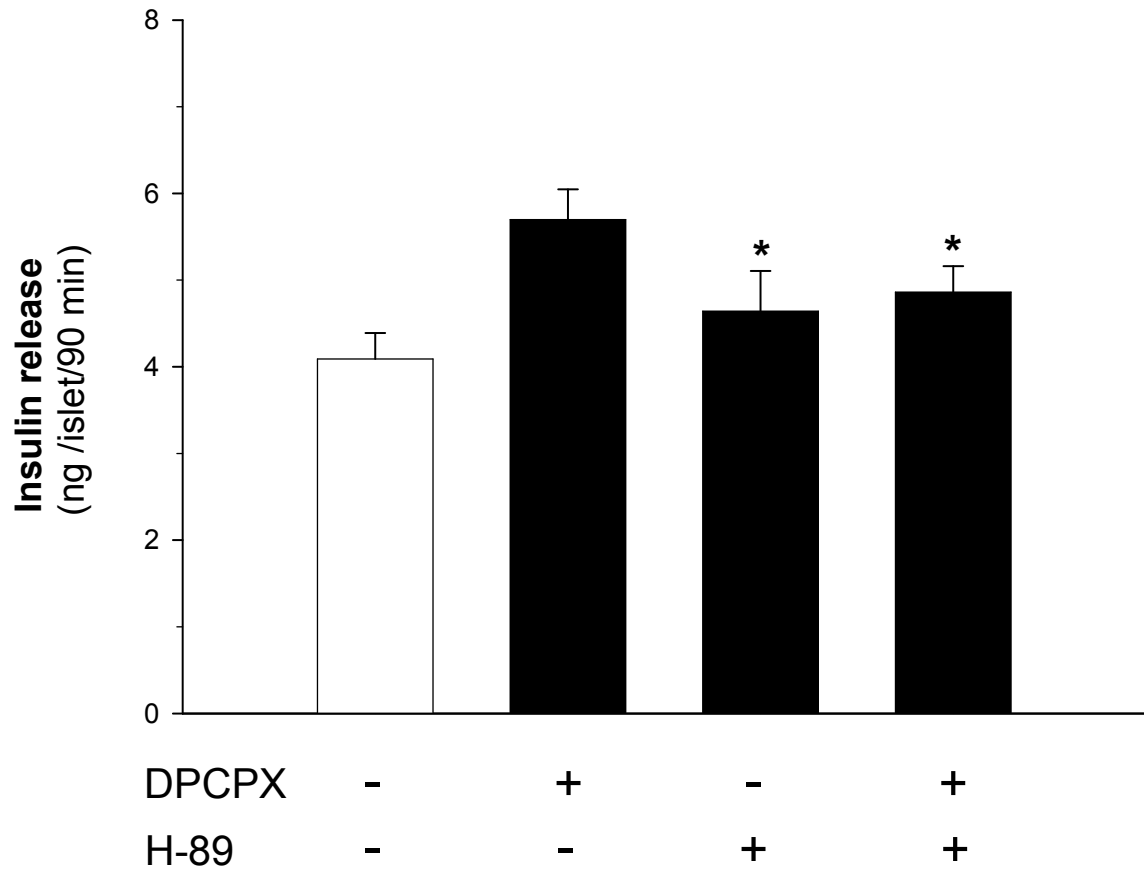
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