Effects of adenosine A_1 receptor antagonism on lipogenesis and lipolysis in isolated rat adipocytes.

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Short title: Adenosine and adipocyte metabolism

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Summary

Adenosine is secreted from adipocytes, binds to adenosine A_1 receptor and modulates various functions of these cells. In the present study, the effects of an adenosine A_1 receptor antagonist (DPCPX; 0.01, 0.1 and 1 μM) on lipogenesis, glucose transport, lipolysis and the antilipolytic action of insulin were tested in rat adipocytes. DPCPX had a very weak effect on lipogenesis and did not significantly affect glucose uptake.

In adipocytes incubated with 1 μM DPCPX, lipolysis increased. This effect was blunted by insulin and by a direct inhibitor of protein kinase A. Moreover, 0.1 μM DPCPX substantially enhanced the lipolytic response to epinephrine and increased cAMP in adipocytes. However, DPCPX was ineffective when lipolysis was stimulated by direct activation of protein kinase A. Adipocyte exposure to epinephrine and insulin with or without 0.1 μM DPCPX demonstrated that this antagonist increased the release of glycerol. However, despite the presence of DPCPX, insulin was able to reduce lipolysis.

It is concluded that DPCPX had a weak effect on lipogenesis, whereas lipolysis was significantly affected. The partial antagonism of adenosine A_1 receptor increased lipolysis in cells incubated with epinephrine alone and epinephrine with insulin due to the synergistic action of 0.1 μM DPCPX and epinephrine.

Key words: adipocytes, lipogenesis, lipolysis, adenosine

Introduction

The cells of white fat tissue, called adipocytes, are responsible for lipid synthesis, release and storage in the organism. Moreover, the endocrine activity of adipocytes is recently well established (Rondinone 2006). The metabolism and endocrine activity of adipocytes are regulated by numerous factors, including hormones (Londos *et al.* 1999) and dietary compounds (Kandulska and Szkudelski 1998, Szkudelski and Szkudelska 2000, Lynch *et al.* 2006, Szkudelska *et al.* 2008). Adenosine formed in adipocytes plays an important role in this regulation. The nucleoside is released from the cell, binds to the adenosine A_1 receptor and modulates numerous intracellular processes. It is generally believed that adenosine inhibits lipolysis and enhances the lipogenic and antilipolytic action of insulin. The stimulatory effects of the nucleoside on insulin-induced glucose transport and metabolism are also known (Schwabe *et al.* 1974, Smith *et al.* 1984). Furthermore, adenosine is involved in the regulation of leptin secretion (for review see Szkudelski 2007). It was demonstrated that adenosine receptor agonist increased blood leptin in the rat (Rice *et al.* 2000) and enhanced hormone secretion from isolated adipocytes, whereas removal of endogenous adenosine or blocking of adenosine A_1 receptor diminished secretion of leptin (Cheng *et al.* 2000).

The release of adipocyte-derived adenosine may be disturbed as a result of changes in the activity of enzymes engaged in adenosine metabolism - 5'-nucleotidase, adenosine deaminase and adenosine kinase (Jamal and Saggerson 1987). This effect was observed, among others, in streptozotocin-induced diabetic rats in which the activity of 5'-nucleotidase and adenosine deaminase was increased (Jamal and Saggerson 1987, Koopmans *et al.* 1989). The action of adenosine may be also disturbed as a result of altered cellular sensitivity to the nucleoside (Green and Johnson 1991, Saggerson *et al.* 1991).

Data from the literature point to the important role of the proper action of adenosine in preventing obesity. In the obesity, the adenosine A_1 receptor signalling pathway was found to be disturbed. The increased activity of adenosine A_1 receptor was observed in adipocytes isolated from obese

animals (LaNoue and Martin 1994). Moreover, in fat cells of Zucker rats, lipolytic hormones were less effective in stimulating cAMP production, whereas the sensitivity to the inhibition of lipolysis by the adenosine agonist was increased (Vannucci *et al.* 1989). The disturbed adenosine action was also shown in adipocytes isolated from obese humans (Kaartinen *et al.* 1991). On the other hand, overexpression of the adenosine A_1 receptor in adipose tissue appeared to protect mice on high fat diet from insulin resistance (Dong *et al.* 2001). The improved sensitivity to insulin was also recently demonstrated in rats on high fat diet treated with a selective adenosine A_1 receptor agonist (Dhalla *et al.* 2007). In the other study, the adenosine receptor agonist normalized insulin sensitivity in adipocytes obtained from subjects with polycystic ovary syndrome (Ciaraldi *et al.* 1997). Takasuga *et al.* (1999) demonstrated that adenosine decomposition or adenosine A_1 receptor inhibition diminishes the insulin-induced accumulation of phosphatidylinositol 3,4,5-triphosphate in adipocytes, whereas adenosine A_1 receptor agonist exerts the opposite effect. The important role of adenosine in the action of insulin is additionally confirmed by the most recent results indicating that adenosine A_1 receptor agonists may be useful in the treatment of type 2 diabetes (Dhalla *et al.* 2008).

It is well established that the regulatory role of adenosine is affected by fasting. Lipolysis induced by adenosine decomposition (Ben Cheikh *et al.* 1994) or by adenosine A_1 receptor blockade (Szkudelski *et al.* 2004) was found to be higher in adipocytes obtained from fasted rats. Moreover, Chohan *et el.* (1984) revealed that fat cells isolated from fasted rats and exposed to adenosine deaminase and epinephrine were less sensitive to the antilipolytic action of the non-hydrolysable adenosine analogue (PIA). It was also revealed that, in the presence of adenosine deaminase, the lipolytic response to glucagon was substantially enhanced in adipocytes of fasted rats compared with cells obtained from fed animals (Honnor and Saggerson 1980).

The role of adenosine in the regulation of adipocyte functions seems to be underestimated and, in many experiments, this important pathway is omitted. In the present study, the effects of DPCPX,

an adenosine A_1 receptor antagonist, on lipogenesis, glucose transport, lipolysis and the antilipolytic action of insulin were investigated in freshly isolated rat adipocytes to put more light on the regulatory role of adenosine in these cells.

Methods

Animals

Male Wistar rats that weighed 200-250 g and purchased from Brwinow (Poland) were used in all experiments. The rats were fed a standard laboratory diet ad libitum (Labofeed B, 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 of 21 ±1°C and were killed by decapitation. The experimental protocols were approved by the Local Ethical Commission for Investigation on Animals.

Isolation of adipocytes

Adipocytes were isolated from the epididymal fat tissue according to the method described by Rodbell (1964) with minor modifications (Szkudelska *et al.* 2000). In each experiment, the tissue obtained from several rats was pooled, rinsed with 0.9% NaCl, cut down into pieces and placed in a plastic flask with Krebs-Ringer buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$; pH=7.4) containing 3 mM glucose, 3% bovine serum albumin (BSA), 10 mM HEPES and 2 mg/ml collagenase. The buffer was vigorously gassed for about 15 min with 95% O_2-5% CO_2. The same buffer was used in each experiment. The incubation with collagenase was carried out for 90 min in a water bath at 37 °C with gentle shaking. Isolated cells were filtered through nylon mesh, rinsed with warm (37 °C) Krebs-Ringer buffer without collagenase and counted under the microscope with Bürker-Türk counting chamber.

Lipogenesis

In each experiment, isolated adipocytes (10^6 cells/ml) were transferred into plastic tubes with Krebs-Ringer buffer (pH=7.4) containing 3 mM glucose, 0.5 μCi of [U-^{14}C]glucose, 3% BSA and 10 mM HEPES and were incubated for 90 min in a water bath at 37 °C with gentle shaking. The effect of DPCPX on insulin-stimulated glucose conversion to lipids was determined when adipocytes were exposed to 0.01, 0.1 and 1 μM DPCPX in the presence of 1 or 10 nM insulin. Each treatment was performed in four replications (i.e. four tubes incubated simultaneously). At the end of each incubation, the reaction was stopped by addition of Dole's extraction mixture (Dole and Meinertz 1960). After shaking, H_2O and heptane were added. Tubes were shaken once again, samples of the upper phase were transferred into counting vials containing scintillation cocktail and total lipid radioactivity was measured.

Glucose uptake

2-deoxy-D-[1-^{3}H]-glucose uptake was determined as described previously with minor modifications (Zmuda-Trzebiatowska *et al.* 2006). Adipocytes were preincubated for 10 min in Krebs-Ringer buffer containing 3 mM glucose alone, glucose with 10 nM insulin and glucose, insulin and 1 μM DPCPX in a water bath at 37 °C with gentle shaking. After preincubation, 2 μCi of 2-deoxy-D-[1-^{3}H]-glucose was added to each tube and cells were incubated for additional 5 min. Each treatment was performed in quadruplicates. Afterwards, adipocytes were separated immediately from the buffer by centrifugation through silicone oil and the radioactivity was measured.

Lipolysis

In each experiment, isolated cells (10^6 cells/ml) were transferred into plastic tubes with Krebs-Ringer buffer (pH=7.4) containing 3 mM glucose, 3% BSA and 10 mM HEPES and were incubated for 90 min in a water bath at 37 °C with gentle shaking. The effect of DPCPX on basal triglyceride breakdown was determined when adipocytes were incubated with 0.01, 0.1 and 1 μM DPCPX without any hormones. It was also determined whether the lipolytic action of DPCPX is inhibited by the main physiological antilipolytic agent – insulin, and by a direct inhibitor of protein kinase A – H-89. In these studies, fat cells were exposed to 0.1 and 1 μM DPCPX with or without 10 nM insulin or 50 μM H-89.

In the further investigations, the effect of DPCPX on the lipolytic action of epinephrine and dibutyryl-cAMP (DB-cAMP, a direct activator of protein kinase A) was compared. Isolated cells were stimulated by 0.125, 0.25 and 0.5 μM epinephrine or by 0.125, 0.25 and 0.5 mM DB-cAMP in the absence or presence of 0.1 μM DPCPX.

In order to determine the influence of DPCPX on the antilipolytic action of insulin, fat cells were incubated in the buffer containing 0.5 μM epinephrine alone or 0.5 μM epinephrine plus 1 or 10 nM insulin with or without 0.1 μM DPCPX. In the further studies, insulin was replaced by 50 μM H-89 and the antilipolytic activity of this agent on epinephrine-induced lipolysis was tested in the absence or presence of 0.1 μM DPCPX.

Each treatment was performed in quadruplicates. At the end of each incubation, adipocytes were aspirated and the quantity of glycerol released from cells to the incubation buffer was determined (Foster and Dunn 1973).

cAMP measurement

To test the effect of DPCPX on cAMP concentrations, the isolated adipocytes were transferred to plastic tubes with Krebs-Ringer buffer (pH=7.4) containing 3 mM glucose, 3% BSA and 10 mM HEPES. The final amount of fat cells was 10^4 per 180 μl. The adipocytes

were incubated with 1 μM DPCPX, 0.25 μM epinephrine or with 0.25 μM epinephrine and 0.1 μM DPCPX. Control incubations with non-treated cells were also performed. After 30 min, 20 μl of the lysis buffer was added to each tube, the mixture was shaken and incubated for 10 min at room temperature. Afterwards, 100 μl of cell lysate was transferred to the assay plate and total cAMP was measured using non-acetylation EIA procedure according to the instruction enclosed by the manufacturer.

Reagents

Insulin (from bovine pancreas), DPCPX, epinephrine, DB-cAMP, collagenase (from Clostridium histolyticum, for adipocyte isolation) and all reagents used to prepare Krebs-Ringer buffer and to determine glycerol were obtained from Sigma. Dimethyl sulfoxide and H-89 were from ICN Pharmaceuticals, Inc., whereas [U-^{14}C]glucose (specific activity 9.80 GBq/mmol) was from New England Nuclear Research Products. cAMP kit (EIA) and 2-deoxy-D-[1-^{3}H]-glucose (specific activity 296 GBq/mmol) were purchased from Amersham. Scintillation cocktail (Hi Safe, OptiPhase) was from Perkin Elmer. DPCPX and H-89 stock solutions were prepared in dimethyl sulfoxide.

Statistics

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

Lipogenesis

Insulin-stimulated glucose conversion to lipids was substantially enhanced compared with lipogenesis found in the absence of insulin. Lipogenesis induced by 1 nM insulin was not significantly affected in adipocytes exposed to 0.01 (data not shown), 0.1 and 1 μM DPCPX (Fig. 1). In fat cells stimulated by 10 nM insulin, lipogenesis was elevated compared with 1 nM insulin. However, 0.01 (data not shown) and 0.1 μM DPCPX had no influence on glucose conversion to lipids in isolated rat adipocytes, whereas 1 μM DPCPX significantly increased lipogenesis induced by 10 nM insulin (Fig. 1).

Glucose uptake

Adipocyte exposure to 10 nM insulin significantly increased glucose uptake compared with cells incubated without this hormone. It was found that 1 μM DPCPX tended to reduce insulin-induced glucose uptake, however, the diminution was not statistically significant (Fig. 2).

Lipolysis

Non-stimulated lipolysis was not changed by 0.01 μM DPCPX (data not shown). Basal triglyceride breakdown measured in the presence of 0.1 μM DPCPX tended to be elevated, but the rise was not statistically significant. Insulin (10 nM) did not affect lipolysis in adipocytes incubated with 0.1 μM DPCPX, whereas H-89 reduced this process (Fig. 3). In adipocytes exposed to 1 μM DPCPX, glycerol release increased dramatically compared with non-treated cells. The lipolytic effect of 1 μM DPCPX was substantially blunted in the presence of 10 nM insulin. It was also demonstrated that H-89 exerted profound, greater than insulin, inhibitory influence on lipolysis induced by 1 μM DPCPX. However, both antilipolytic agents did not reduce DPCPX-induced lipolysis to the basal level (Fig. 3).

The next set of experiments was carried out to test whether triglyceride breakdown induced by epinephrine and DB-cAMP is affected by DPCPX. In these investigations, 0.1 μM DPCPX was

used since 1 μM DPCPX alone was able to induce lipolysis. Incubations of adipose cells with increasing concentrations of epinephrine (0.125 – 0.5 μM) allowed demonstration that 0.1 μM DPCPX substantially enhanced epinephrine-induced glycerol release. The effect of DPCPX was greater at lower (0.125 μM) than at higher concentration (0.5 μM) of epinephrine (Fig. 4). It was also shown that 0.1 μM DPCPX failed to significantly alter the lipolytic response of adipocytes to DB-cAMP (0.125 – 0.5 mM; Fig. 5).

In the further part of the study, the effect of DPCPX on the antilipolytic action of insulin and H-89 was tested. The obtained results revealed that 1 and 10 nM insulin substantially reduced epinephrine-induced lipolysis. However, in the presence of 0.1 μM DPCPX, the release of glycerol was higher than in adipocytes incubated with epinephrine and insulin without DPCPX. The similar effect was found in the case of H-89 (Fig. 6).

cAMP concentrations

Incubations of adipocytes for 30 min with 1 μM DPCPX resulted in enhanced concentration of cAMP compared with non-treated cells. The exposure of fat cells to 0.25 μM epinephrine also increased cAMP. However, in the latter case, the observed effect was substantially greater. It was also revealed that in adipocytes stimulated with 0.25 μM epinephrine and exposed to 0.1 μM DPCPX, cAMP was dramatically enhanced compared with cells incubated with epinephrine alone (Tab. 1).

Discussion

Adipocyte-derived adenosine is thought to enhance insulin action in fat cells. However, the role of this nucleoside in lipogenesis is not well established and some discrepancies in the literature can be found. In the present investigations, the effects of a specific adenosine A_1 receptor antagonist,

DPCPX, on lipogenesis in isolated rat adipocytes were studied for the first time. These experiments revealed that DPCPX failed to affect lipogenesis induced by 1 nM insulin. Similarly, glucose conversion to lipids stimulated by 10 nM insulin was unchanged in cells exposed to 0.01 and 0.1 μM DPCPX. However, 1 μM DPCPX enhanced lipogenesis induced by 10 nM insulin without significant changes in glucose uptake. Heseltine *et al.* (1995) also observed that removal of adenosine had only a slight influence on insulin-induced glucose transport. Moreover, Okuda (2003) demonstrated the stimulatory action of 0.01 and 0.1 mM adenosine on insulin-induced lipogenesis, whereas 1 mM adenosine was completely ineffective. It was also found that in adipocytes incubated with adenosine deaminase (to decompose adipocyte-derived adenosine), the maximal influence of insulin on glucose oxidation and lipogenesis was not changed. However, under these conditions, addition of adenosine increased the sensitivity of fat cells to insulin (Goren *et al.* 1986). These data and results of our present experiment suggest that the attenuation of adenosine action by its decomposition or by adenosine A_1 receptor blockade has a slight influence on insulin-induced glucose conversion to lipids in rat adipocytes. This is in contrast to insulin-induced leptin secretion since Cheng *et al.* (2000) demonstrated a clear-cut inhibitory effect of DPCPX (0.01, 0,1 and 1 μM) on this process in isolated fat cells.

Conversely to lipogenesis, lipolysis and the antilipolytic action of insulin appeared to be more sensitive to DPCPX. In our study, 0.01 and 0.1 μM DPCPX failed to enhance basal glycerol release, however, in cells exposed to 1 μM DPCPX, glycerol release markedly increased. A similar increase in basal lipolysis has been found in adipocytes with adenosine A_1 receptor deficiency (Johansson *et al.* 2007). This is in accord to the notion that endogenous adenosine causes tonic inhibition of lipolysis (Liang *et al.* 2002). Interestingly, lipolysis evoked by adenosine A_1 receptor antagonism appeared to be substantially restricted by insulin and H-89 (a direct inhibitor of PKA). A similar inhibitory effect of insulin was observed previously when lipolysis was induced by adenosine decomposition (Tebar *et al.* 1996). The ability of insulin to counteract lipolysis bring

about by 1 μM DPCPX indicates that this antagonist increases cAMP in adipocytes since insulin does not diminish lipolysis induced without a rise in cAMP (Morimoto *et al.* 1998). The rise in cAMP concentration was indeed demonstrated in adipocytes exposed to 1 μM DPCPX. The obtained results imply that in fat cells in which the inhibitory action of intracellular adenosine on lipolysis is aggravated, insulin can partially compensate for this effect, thus preventing exaggerated release of glycerol and free fatty acids. This may have physiological relevance since Johansson *et al.* (2007) demonstrated that eliminating adenosine A_1 receptor does not generate in adipocytes any compensatory mechanisms of diminishing lipolysis.

Results of our present study revealed that 0.1 μM DPCPX effectively potentiated the lipolytic response to epinephrine in adipocytes. The adenosine A_1 receptor antagonist at the same concentration failed to increase basal glycerol release indicating rather synergistic than additive action of epinephrine and DPCPX. It is difficult to evaluate at what extent 0.1 μM DPCPX blocked the interaction of adenosine with its receptor. However, the lack of influence of 0.1 μM DPCPX on basal glycerol release allows supposition that the blockade of adenosine action was only partial. Interestingly, the effect of 0.1 μM DPCPX on epinephrine-induced triglyceride breakdown appeared to be greater at lower concentrations of the hormone. This demonstrates that the lipolytic effectiveness of epinephrine acting at low concentrations may be substantially enhanced when adenosine action in adipocytes is only partially diminished.

Conversely to epinephrine-induced lipolysis, the lipolytic response to DB-cAMP, a direct activator of PKA, was not significantly increased by 0.1 μM DPCPX. This indicates that DPCPX does not exert synergistic action with all lipolytic agents and implies that the partial blockade of adenosine A_1 receptor potentiates epinephrine-induced lipolysis via changes upstream of PKA in the lipolytic cascade. Then, the activation of steps in the lipolytic cascade upstream of PKA is a prerequisite for enhanced lipolysis found in the presence of 0.1 μM DPCPX. This assumption is confirmed by our observation that the increase in epinephrine-induced lipolysis evoked by 0.1 μM DPCPX was

accompanied by a substantial rise in cAMP. This is in accord to the notion that the inhibitory action of adenosine on lipolysis in adipocytes is due to reduced activity of adenylyl cyclase (Londos *et al.* 1978).

Inhibition of lipolysis is one of the pivotal aspects of insulin action in adipocytes. This effect is achieved *via* activation of cAMP phosphodiesterase 3B (Degerman *et al.* 1990, Eriksson *et al.* 1995). Deterioration of the antilipolytic action of insulin in fat tissue results in increased concentrations of plasma free fatty acids, contributing to insulin resistance in the other target tissues, such as liver and skeletal muscle, and to develop type 2 diabetes (Arner 2003). The effectiveness of insulin to counteract lipolysis is known to be influenced by numerous factors (Ibrahim *et al.* 2005, Szkudelska *et al.* 2008). In the present study, the partial blockade of adenosine action by 0.1 μM DPCPX substantially enhanced glycerol release from fat cells incubated with epinephrine and 1 nM insulin. This effect was not mitigated by high (10 nM) insulin concentration. However, the comparison of lipolysis in adipocytes exposed to epinephrine and DPCPX with lipolysis in cells incubated with epinephrine, DPCPX and insulin revealed that in the presence of insulin glycerol release was substantially diminished. This clearly demonstrates that the antilipolytic action of insulin was preserved despite the partial blockade of adenosine A_1 receptor. Then, the observed rise in glycerol release induced by DPCPX resulted from the amplifying influence of DPCPX on epinephrine action, not from the inability of insulin to counteract lipolysis. Similarly to insulin, H-89 was also able to reduce triglyceride breakdown indicating that lipolysis induced by epinephrine and DPCPX may be effectively attenuated by pharmacological inhibition of PKA.

In conclusion, results obtained in the present study demonstrated that the blockade of adenosine A_1 receptor by DPCPX had a slight effect on glucose conversion to lipids, whereas glycerol release from adipocytes was substantially affected. The partial blockade of adenosine A_1 receptor by DPCPX enhanced lipolysis induced by epinephrine. The similar effect of this antagonist was

shown in fat cells incubated with epinephrine and insulin, although, the antilipolytic action of insulin was preserved.

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Figure legends:

Fig. 1. The effect of DPCPX on lipogenesis stimulated by 1 (grey bars) or 10 (black bars) nM insulin in isolated rat adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Means marked by different letters differ statistically at $P<0.05$. Open bar - non-stimulated lipogenesis.

Fig. 2. The effect of 1 μM DPCPX on glucose uptake stimulated by 10 nM insulin in isolated rat adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Means marked by different letters differ statistically at $P<0.05$. Open bar - non-stimulated glucose uptake.

Fig. 3. The effect of 0.1 (grey bars) or 1 (black bars) μM DPCPX on lipolysis in isolated rat adipocytes and the influence of 10 nM insulin or 50 μM H-89. Open bar represents lipolysis in non-treated adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Means marked by different letters differ statistically at $P<0.05$. Open bar - lipolysis in non-treated adipocytes.

Fig. 4. The effect of DPCPX on epinephrine-induced lipolysis in isolated rat adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Means marked by asterisks differ statistically at $P<0.05$ between the appropriate concentrations of epinephrine with or without DPCPX. Black circles – epinephrine alone, open circles – epinephrine with 0.1 μM DPCPX.

Fig. 5. The effect of DPCPX on dibutyryl-cAMP (DB-cAMP)-induced lipolysis in isolated rat adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Black circles – DB-cAMP alone, open circles – DB-cAMP with 0.1 μM DPCPX.

Fig. 6. The effect of DPCPX on the inhibitory action of insulin (1 or 10 nM) and H-89 (50 μM) on epinephrine-induced lipolysis in isolated rat adipocytes. Values represent means ±SEM of 12 determinations from three separate experiments. Means marked by different letters differ statistically at $P<0.05$. Grey bars – 0.25 μM epinephrine, black bars – epinephrine with 0.1 μM DPCPX.

Table 1. The effect of DPCPX, an adenosine A_1 receptor antagonist, on cAMP concentrations in rat adipocytes.

Experimental conditions	cAMP (fmol/10^4 cells/30 min)
Non-treated	5.716 ±1.00
DPCPX 1 μM	15.55 ±2.25
Epinephrine 0.25 μM	258.0 ±77.4
Epinephrine 0.25 μM + DPCPX 0.1 μM	2034 ±132

Values represent means ±SEM of 12 determinations from three separate experiments. All values differ statistically at $P<0.05$.

List of abbreviations

cAMP – adenosine 3',5'-cyclic monophosphate

DB-cAMP – dibutyryl-cAMP (N^6,2'-O-dibutyryladenosine 3': 5'-cyclic monophosphate); protein kinase A activator

DPCPX - 8-cyclopentyl-1,3-dipropylxantine; A_1 adenosine receptor antagonist

H-89 - N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide; protein kinase A inhibitor

PIA - N^6-($_L$-2-phenylisopropyl)adenosine; A_1 adenosine receptor agonist

PKA – protein kinase A

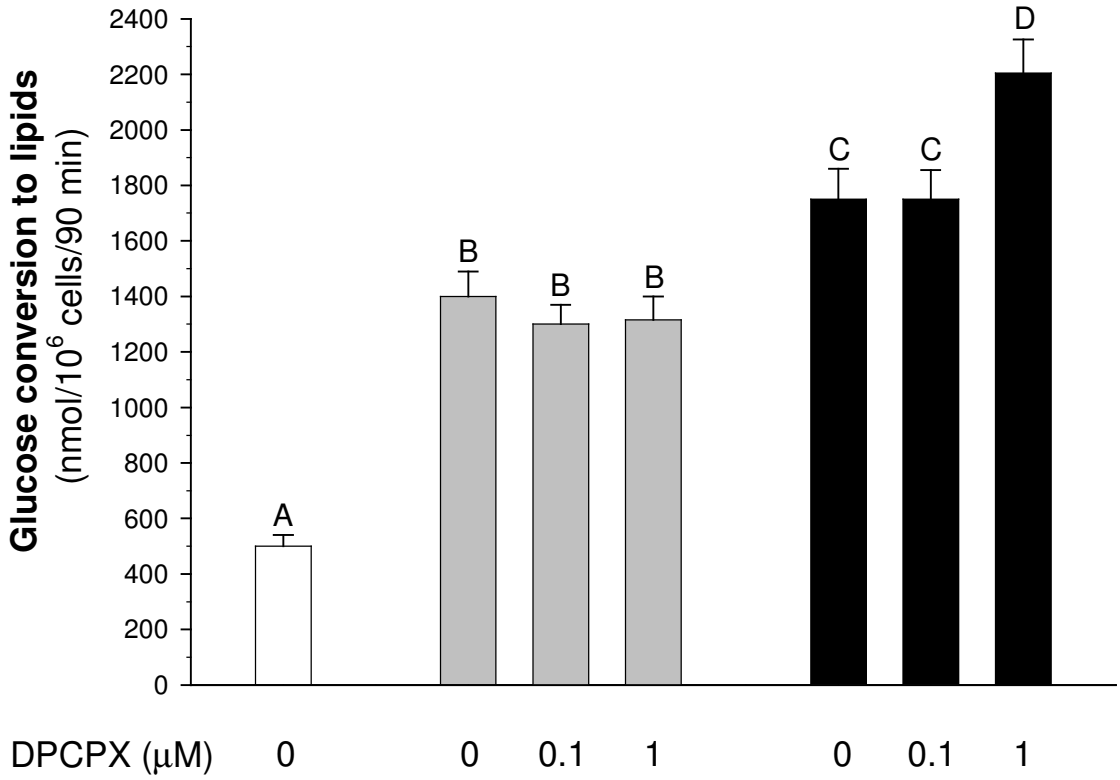
Glucose conversion to lipids
(nmol/10^6 cells/90 min)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
A
B
B
B
C
C
D
DPCPX (μM)
0
0
0.1
1
0
0.1
1

Fig. 1.

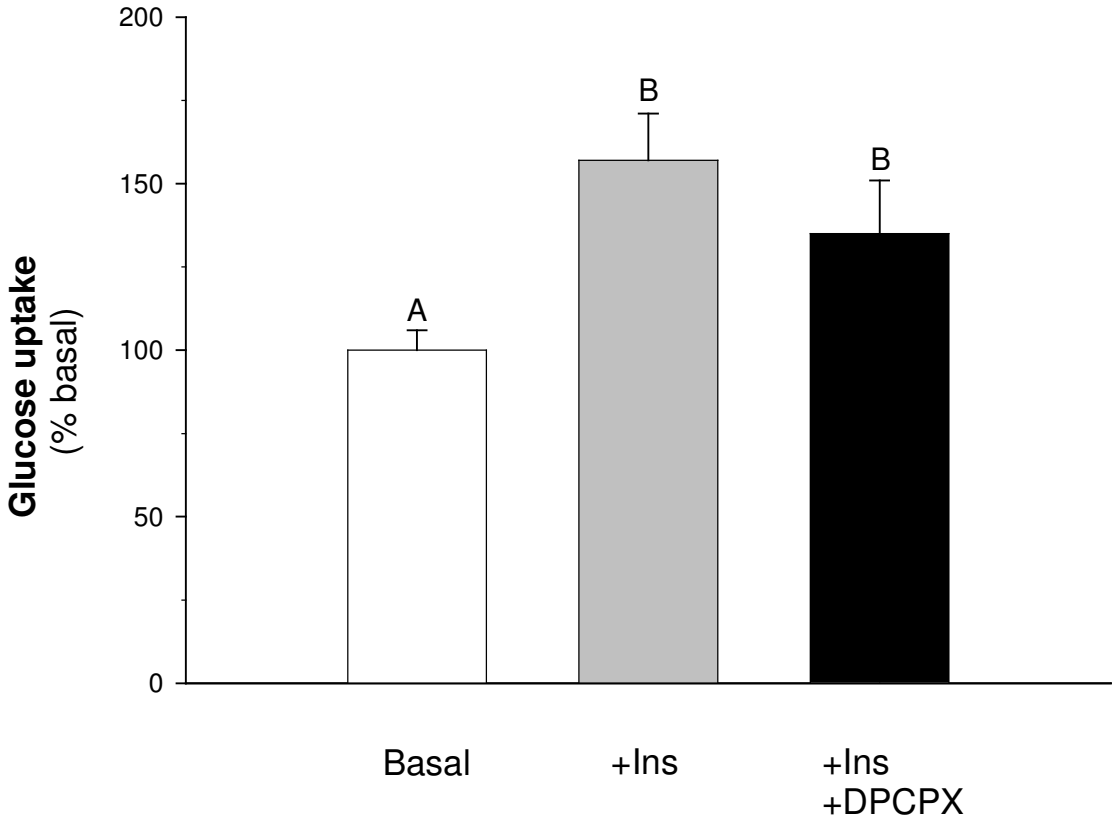

Fig. 2.

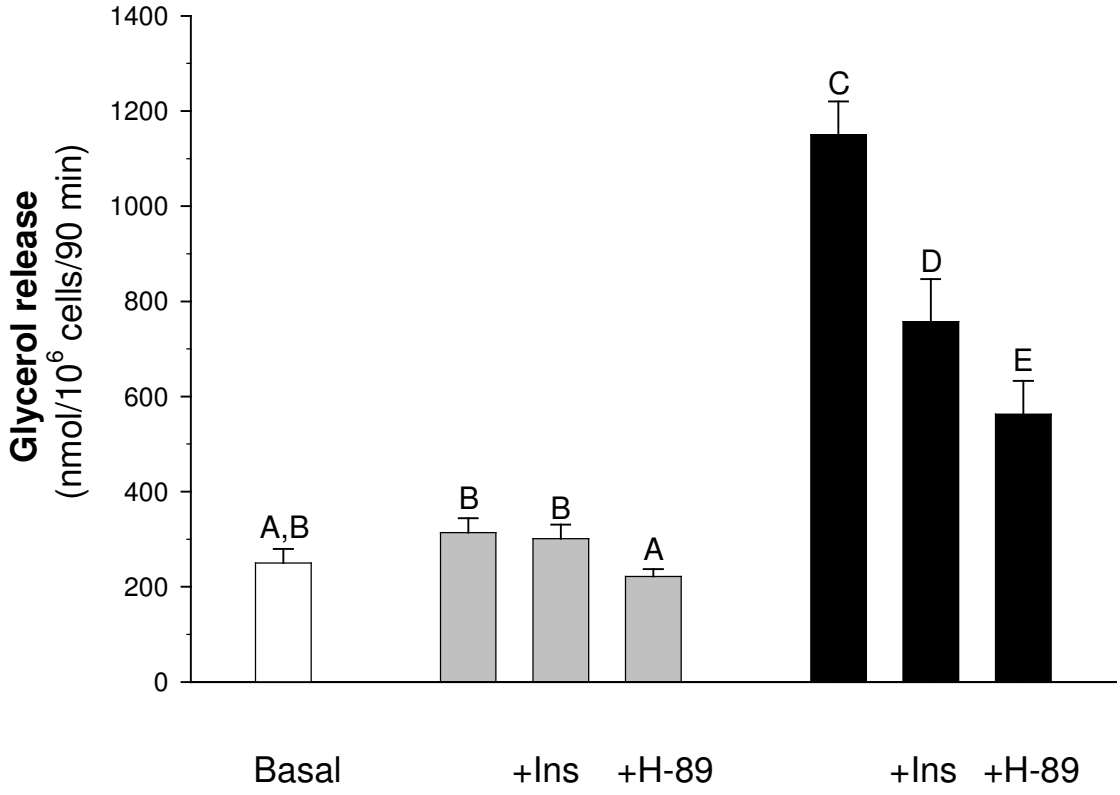
Glycerol release
(nmol/10^6 cells/90 min)
1400
1200
1000
800
600
400
200
0
A,B
B
B
A
C
D
E
Basal
+Ins
+H-89
+Ins
+H-89

Fig. 3.

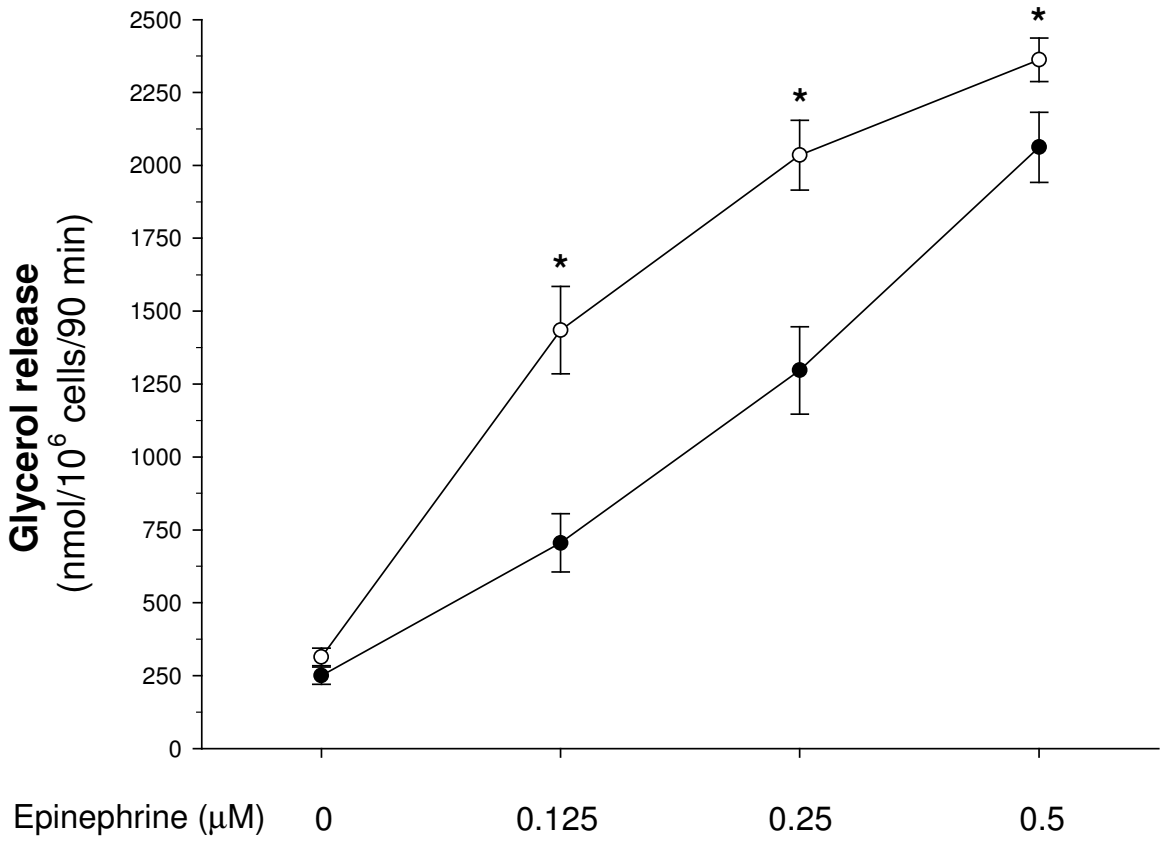
Glycerol release
(nmol/10^6 cells/90 min)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
*
*
*
Epinephrine (μM)
0
0.125
0.25
0.5

Fig. 4.

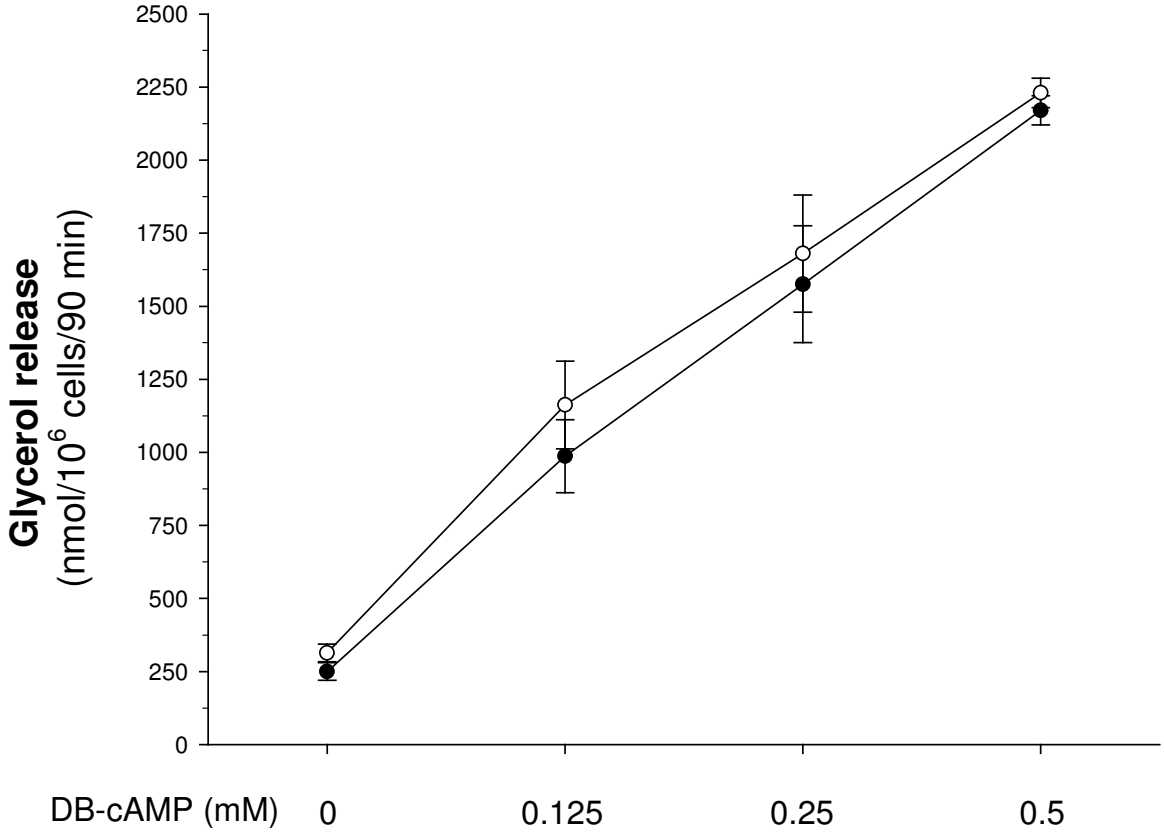
Glycerol release
(nmol/10^6 cells/90 min)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
DB-cAMP (mM)
0
0.125
0.25
0.5

Fig. 5.

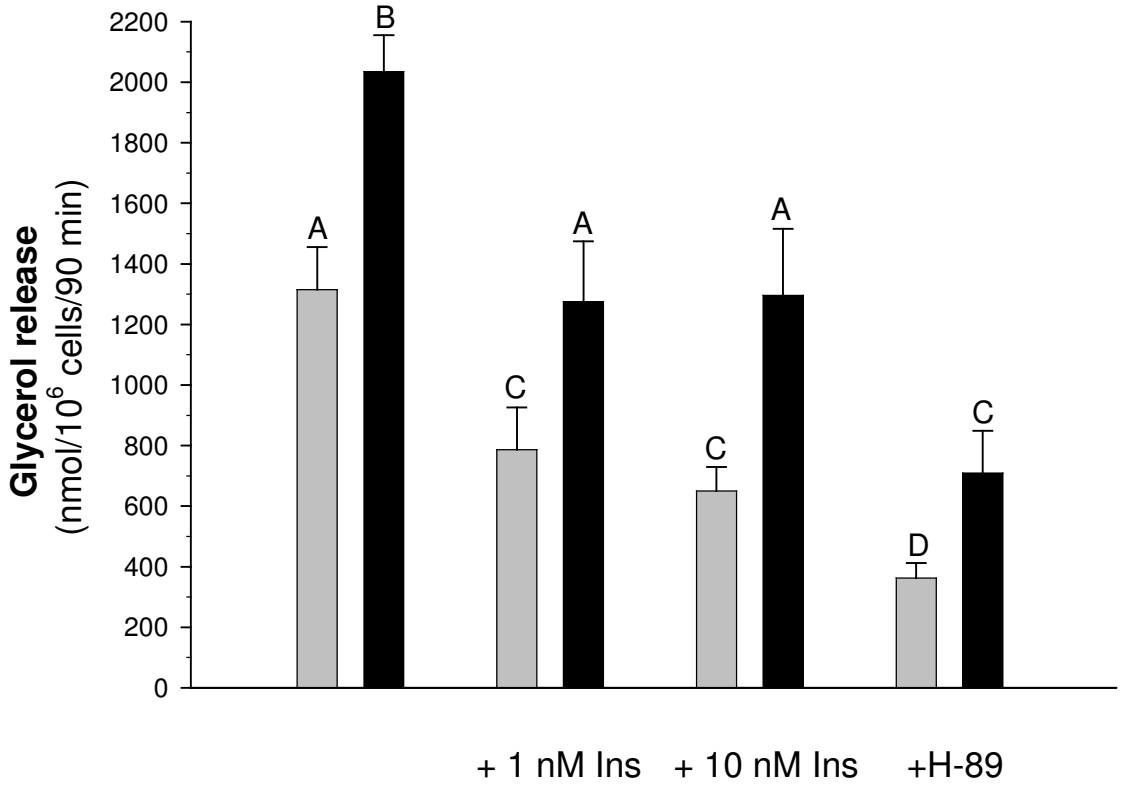
Glycerol release
(nmol/10^6 cells/90 min)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
A
B
C
A
C
A
D
C
+ 1 nM Ins
+ 10 nM Ins
+H-89

Fig. 6.