The inhibitory GTP-binding protein (G_i) regulates the agonistic property of β -adrenergic ligands in isolated rat adipocytes

Evidence for a priming effect of cyclic AMP

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Prenalterol, an allegedly β_1 -selective adrenergic agonist with high intrinsic sympathomimetic activity (ISA), was shown to be weakly lipolytic in rat adipocytes. However, in pertussis-toxin-treated adipocytes, the ISA of prenalterol was markedly increased (from 10–20% to approx. 100% of that of isoprenaline). The cellular sensitivity was also increased (EC_{50} approx. 60 nM and approx. 3 μ M in pertussis-toxin-treated and control cells respectively). A similar effect was seen for other partial agonists such as the β_2 -selective agonist terbutaline and for β -adrenergic antagonists with some intrinsic activity (metoprolol, pindolol). There was no clear change in sensitivity to isoprenaline's ability to stimulate adenylate cyclase in adipocyte membranes from pertussis-toxin-treated animals but the cyclase activity was increased approx. 4-fold in the presence of 1 μ M-GTP. Prenalterol stimulated lipolysis by only small increases in intracellular cyclic AMP (cAMP) levels (less than 10% of that seen with isoprenaline). Basal lipolysis was increased in cells from pertussis-toxin-treated rats and the cellular sensitivity to the non-degradable cAMP analogue, N^6 -monobutyryl-cAMP, was increased. In control cells, a submaximal concentration of prenalterol (0.1 μ M) increased the sensitivity to the cAMP analogues, N^6 -monobutyryl-cAMP and 8-bromo-cAMP. A low concentration (1 mM) of 8-bromo-cAMP also increased the effect of prenalterol. Similar effects were seen when the phosphodiesterase was inhibited. Thus (1) lipolysis is extremely sensitive to small increases in intracellular cAMP; (2) the degree of activation of adenylate cyclase and thus cAMP formation is the rate-limiting step for the biological response of partial agonists; (3) the inhibitory GTP-binding protein, G_i , is an important modulator ('tissue factor') of the β -adrenergic agonistic property; (4) low levels of cAMP exert a priming effect on protein kinase A.

INTRODUCTION

Prenalterol was first described as a β_1 -selective adrenergic agonist with high intrinsic sympathomimetic activity (ISA) in both experimental animals (Carlsson *et al.*, 1977) and man (Rönn *et al.*, 1979; Scott *et al.*, 1979; Ariniago *et al.*, 1979). However, binding studies have shown (Minneman *et al.*, 1979; Hedberg & Mattsson, 1981) that prenalterol binds with equal affinity to β_1 - and β_2 -adrenoceptors. The extensive studies by Kenakin and co-workers (Kenakin & Beek, 1980; Kenakin, 1982) showed that the relative β_1 -selectivity of prenalterol is due to its low intrinsic efficacy together with differences in the stimulus-response relationship in various organs. Thus post-receptor 'tissue factors', as yet unidentified, are important for the β -adrenergic effect and consequently for the tissue sensitivity, selectivity and responsiveness to partial agonists.

Adenylate cyclase is bimodally regulated (Casey & Gilman, 1988) by agonist binding to receptors interacting with the GTP-binding stimulatory (G_s) and inhibitory (G_i) coupling proteins. The amount and activity of these proteins are regulated by both genetic (Farfel *et al.*, 1980) and hormonal (Ros *et al.*, 1988) factors. Differences in these proteins in various tissues may thus be important regulators of the stimulus-response relationships.

Pertussis toxin inhibits G_i -protein by irreversibly ADP-ribosylating the α -subunit (Murayama & Ui, 1983). In the present study we examined the importance of G_i -protein as a potential 'tissue factor' for the β -adrenergic stimulus-response relationship in adipose cells when stimulated by different partial and full agonists as well as cyclic AMP (cAMP) analogues. We also studied the relationship between intracellular cAMP levels and

lipolysis and obtained evidence for a priming effect of cAMP on protein kinase A (PKA).

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MATERIALS AND METHODS

Materials

Collagenase, (–)-isoprenaline, DL-propranolol, 3-isobutyl-1-methylxanthine (IBMX), 8-bromo-cAMP, N^6 -monobutyryl-cAMP and BSA (fraction V) were from Sigma Chemical Co., St. Louis, MO, U.S.A. Phosphocreatine, creatine kinase and glutathione were from Boehringer, Mannheim, Germany. Aluminium oxide was from Merck, Darmstadt, Germany and [α - 32 P]ATP (10–50 Ci/mmol) and [2,8- 3 H]cAMP (30–50 Ci/mmol) were from Amersham, Bucks., U.K. [32 P]NAD $^+$ (30 Ci/mmol) was from New England Nuclear. Prenalterol, metoprolol and alprenolol were gifts from Hässle (Mölnådal, Sweden), pindolol was donated by Sandoz (Stockholm, Sweden) and terbutaline by Draco (Lund, Sweden). OPC 3911, a specific inhibitor of the cGMP-inhibited low- K_m particulate cAMP phosphodiesterase, was a gift from Dr. Per Belfrage, University of Lund, Sweden. Medium 199 was obtained from Statens Bakteriologiska Laboratorium (Stockholm, Sweden) and pertussis toxin from List Biological Laboratories, Campbell, CA, U.S.A. The radioimmunoassay kit (RIANEN-cAMP) (125 I) for cAMP was purchased from New England Nuclear.

Cell preparation

Male Sprague-Dawley rats (150–200 g), fed *ad libitum*, were

injected intraperitoneally with either pertussis toxin (3–5 μ g) or 0.9% NaCl. The animals were decapitated after 64–72 h, the epididymal fat-pads excised, minced and incubated at 37 °C in medium 199 containing 4% (w/v) BSA and 0.75 mg of collagenase/ml for 60 min. The isolated cells were filtered through a 250 μ m nylon mesh and washed three times in fresh medium.

Preparation of crude cell membranes

Isolated adipose cells were washed twice in 20 mM-Tris/HCl/1 mM-EDTA/255 mM-sucrose buffer, pH 7.4, resuspended in twice the cell volume of the same buffer and homogenized in a Teflon-pestled glass homogenizer.

The homogenate was centrifuged at 16000 g at -4 °C for 15 min and the solidified fat-cake carefully removed. The supernatant was discarded and the pellet resuspended in the same buffer and centrifuged once before being suspended in buffer. This membrane fraction was stored in liquid nitrogen until use.

Lipolysis

Samples (100 μ l) of the adipocyte suspension, lipocrit around 10%, were added to plastic vials containing 2 ml of medium 199 and the indicated concentrations of the different additions. The incubations were performed for 120 min at 37 °C. Glycerol release to the incubation medium was measured enzymically (Laurell & Tibbling, 1966) or radiometrically (Bradley & Kaslow, 1989) and taken as an index of lipolysis.

ADP-ribosylation by pertussis toxin

The effect of pertussis toxin on the incorporation of [32 P]ADP-ribose from [32 P]NAD $^{+}$ was assayed. Membranes (approx. 400 μ g) were pelleted and resuspended in 500 μ l of incubation buffer containing 100 mM-Tris/HCl, 6 mM-MgCl $_2$, 2 mM-GTP, 10 mM-thymidine, 2.5 mM-ATP, 10 mM-isoniazide and 10 μ M-[32 P]NAD $^{+}$, pH 8.0. Pertussis toxin was activated with 50 mM-dithiothreitol for 30 min at room temperature. After addition of activated pertussis toxin (50 μ g), the membranes were incubated for 90 min at 30 °C. The reaction was stopped by centrifugation at 150000 g for 30 min, washing the pellet with ice-cold buffer and solubilizing it in 200 μ l of a solution containing 62.5 mM-Tris/HCl, 2% (w/v) SDS, 10% (v/v) glycerol and 5% (v/v) 2-mercaptoethanol, pH 6.8, and boiling for 5 min. The proteins (50 μ l) were separated by SDS/PAGE [12% (w/v) gel]. The gels were dried on cellophane and autoradiography was performed for 18 h at -70 °C (Kodak X-OMAT S film 100).

Adenylate cyclase

Adenylate cyclase was assayed essentially as described by Hanoune *et al.* (1977). The assay medium contained 1 mM-ATP and [α - 32 P]ATP (10^6 c.p.m.), 5 mM-MgCl $_2$, 1 mM-glutathione, 1 mM-dibuturyl-cAMP, 25 mM-Tris/HCl, pH 7.6, and an ATP-regenerating system consisting of 20 mM-phosphocreatine and 1 mg of creatine kinase/ml. Membrane protein (20–150 μ g) was added to a final incubation volume of 60 μ l to start the reaction and the incubations were carried out at 30 °C for 15 min.

The reactions were terminated by adding 200 μ l of 1 M-HCl and boiling for 6 min. Samples were then buffered with 200 μ l of 1.5 M-imidazole and applied to aluminium oxide columns. Cyclic AMP was eluted with 3 ml of 10 mM-imidazole, pH 7.6.

Blank values were always lower than 0.005% of the total radioactivity applied. The yield from the columns was calculated from the addition of known amounts of [2,8- 3 H]cAMP.

cAMP

Adipocytes were incubated for 4 min at 37 °C in 1 ml of medium 199 containing 4% (w/v) BSA and the indicated con-

centrations of the different agents. The reactions were stopped by the addition of 1 ml of 20% (v/v) trichloroacetic acid, and the cells disrupted by a 20 s sonication in Soniprep 150 (MSE Scientific Instruments, Crawley, West Sussex, U.K.) followed by centrifugation for 10 min at 2500 g . The supernatants were extracted six times with 2 ml of ethyl acetate, the samples dried and then measured for cAMP using a radioimmunoassay (RIANEN-cAMP).

Protein determination

Protein was measured as described by Lowry *et al.* (1951) with BSA as standard.

RESULTS

ADP-ribosylation of G $_i$ -protein

The ADP-ribosylation of an approx. 43 kDa protein, corresponding to G $_i$ -protein, was studied in isolated adipocyte membranes *in vitro*. Ribosylation was inhibited by prior treatment of the animals with pertussis toxin (Fig. 1) showing that this pretreatment ADP-ribosylates the G $_i$ -protein *in vivo*.

Lipolysis stimulated by isoprenaline and prenalterol

In control cells, EC $_{50}$ for isoprenaline was around 0.1 μ M and the maximal stimulatory effect was seen at 1 μ M (Fig. 2a). Pertussis-toxin-treated cells had elevated basal lipolysis (5–6-fold) and were considerably more sensitive to isoprenaline (EC $_{50}$ 20 nM), whereas the maximal effect of isoprenaline was similar to that in control cells (Fig. 2a).

Prenalterol was only a weak agonist in control cells where maximal lipolysis, at most 20% of that of isoprenaline, was induced at 0.1 mM (Fig. 2b). However, in pertussis-toxin-treated cells the maximal lipolysis in response to prenalterol was markedly enhanced and similar to that of isoprenaline. The dose-response curve also showed a marked increase in sensitivity (EC $_{50}$ 60 nM compared with 3 μ M respectively).

The presence of 1 unit of adenosine deaminase (ADA)/ml in the incubation medium to eliminate adenosine increased both sensitivity and responsiveness to prenalterol but not to the same degree as that seen in pertussis-toxin-treated cells (see Fig. 6b).

Lipolysis stimulated by other partial agonists and antagonists

Terbutaline, a β_2 -adrenergic agonist, is a partial agonist in adipose tissue. In pertussis-toxin-treated cells, both sensitivity and responsiveness to terbutaline were markedly increased (EC $_{50}$ approx. 0.1 μ M and approx. 30 μ M, and maximal response 1220 and 550 nmol/10 6 cells respectively).

Alprenolol and pindolol, both non-specific β -adrenergic antagonists with low ISA, had no stimulatory effect on lipolysis in control cells. However, in pertussis-toxin-treated cells a stimu-

Fig. 1. SDS/PAGE of plasma-membrane proteins

Autoradiography was performed on gels run with proteins from control (lane 2) and pertussis-toxin-treated (lane 3) rats. All membranes were preincubated *in vitro* with pertussis toxin and [32 P]NAD $^{+}$. Lane 1, prestained protein standards. Numbers to the left indicate molecular mass in kDa.

Fig. 2. Effects of isoprenaline, prenalterol and alprenolol on glycerol release

Isolated adipocytes from rats pretreated with pertussis toxin (●, ▲) or 0.9% NaCl (○, △) were incubated with the indicated concentrations of (a) isoprenaline, (b) prenalterol or (c) alprenolol (○, ●) or prenalterol (△, ▲). Results are means of two to three experiments. B, basal (no additions).

Fig. 3. Effects of isoprenaline and prenalterol on lipolysis and cellular cAMP in adipocytes from pertussis-toxin-treated rats

Isolated cells were incubated with the indicated concentrations of isoprenaline (△, □) or prenalterol (▲, ■) and glycerol release (△, ▲) or cellular cAMP (□, ■) was analysed. Data from one representative experiment. B, basal (no additions).

Fig. 4. Relationship between cellular cAMP and glycerol release at various concentrations of isoprenaline or prenalterol

Isolated adipocytes from pertussis-toxin-treated rats were incubated without (○, △) or with (●, ■, ▲) insulin (50 μ -units/ml) and with various concentrations of isoprenaline (5–1000 nM; ○, ●, ■) or prenalterol (0.1–1000 μ M; △, ▲).

Fig. 5. Dose-response curves for the stimulatory effect of isoprenaline on adenylate cyclase in crude adipocyte membranes

Adipocyte membranes from pertussis-toxin-treated (▲, ■) or control (△, □) rats were incubated with the indicated concentrations of isoprenaline in the absence (△, ▲) or presence (□, ■) of 1 μ M-GTP. Results are means of four experiments. B, basal (without isoprenaline).

latory effect of both antagonists was seen (Fig. 2c; results for pindolol not shown). Propranolol, which lacks ISA, had no lipolytic effect in either control or pertussis-toxin-treated cells (results not shown).

cAMP accumulation

cAMP accumulation in control cells stimulated with prenalterol was small and undetectable in our assay. However, a measurable increase in cAMP was seen in cells from pertussis-toxin-treated rats. In the experiment shown in Fig. 3, prenalterol increased cAMP levels 4–5-fold (from 0.6 to 2.7 pmol/10⁵ cells) which stimulated lipolysis to more than 60% of that of isoprenaline. The maximal isoprenaline-stimulated cAMP level was increased more than 100-fold over basal (Fig. 3). Thus the relationship between cAMP levels and lipolysis is not linear, as shown in Fig. 4 where data from different agonist concentrations have been pooled. At low cAMP levels, up to about 5 pmol/10⁵ cells, there is a high sensitivity accounting for approx. 70% of the maximal adipocyte response whereas at higher cAMP levels a further 15-fold increase only stimulated lipolysis another 25–30%, suggesting a marked attenuation in sensitivity to cAMP.

Fig. 6. Effects of IBMX (a) and OPC 3911 and ADA (b) on the stimulatory effect of prenalterol on lipolysis

(a) Effect of prenalterol in the absence (◆) or presence of 3 μM (■), 10 μM (●) or 30 μM (▲) IBMX. (b) Effect of prenalterol in the absence (◆) or presence of 1 μM-OPC 3911 (■), 5 μM-OPC 3911 (●) and 10 μM-OPC 3911 (▲), 1 unit of ADA/ml (○) or 1 unit of ADA/ml + 5 μM-OPC 3911 (□). Results are based on two experiments. B, basal (without prenalterol).

Adenylate cyclase activity

The high GTP concentration used (1 μM) did not further increase basal or isoprenaline-stimulated adenylate cyclase activity in membranes from control cells. The adenylate cyclase activity was increased in membranes from pertussis-toxin-treated cells (Fig. 5). This increase was further accentuated in the presence of 1 μM-GTP, where both basal and isoprenaline-stimulated enzyme activity was increased 2–3-fold. However, EC_{50} for isoprenaline was essentially unchanged after pertussis toxin treatment. No consistent measurable stimulatory effect of prenalterol was seen in membranes from either control or pertussis-toxin-treated cells (results not shown).

The β -adrenergic antagonists inhibited isoprenaline-stimulated adenylate cyclase with the order of potency alprenolol > propranolol > metoprolol = prenalterol (results not shown). Prenalterol at high concentrations almost completely inhibited isoprenaline-stimulated adenylate cyclase activity, which is in accordance with its low stimulatory effect (results not shown).

Priming effect of cAMP on agonistic activity

IBMX increased prenalterol-stimulated lipolysis in control cells in a dose-dependent way (Fig. 6a). A similar but less pronounced effect was seen with the cAMP-inhibited low- K_m phosphodiesterase, with a maximal effect at 5 μM (Fig. 6b).

Addition of 1 unit of ADA/ml enhanced the effect of OPC 3911 (Fig. 6b) as well as that of IBMX (results not shown).

The sensitivity to N^6 -monobutyl-cAMP was increased in cells from pertussis-toxin-treated animals compared with control cells (Fig. 7a). In control cells, a low concentration of prenalterol (0.1 μM) had a similar effect in increasing the sensitivity to both 8-bromo-cAMP (Fig. 7b) and N^6 -monobutyl-cAMP (Fig. 7c). A low concentration of 8-bromo-cAMP (1 mM) also increased the effect of prenalterol (Fig. 7d).

DISCUSSION

The β -adrenergic receptor in both rodent and human adipose cells has repeatedly been characterized as being of mainly β_1 -type (Williams *et al.*, 1976; Jacobsson *et al.*, 1981). In order to elucidate factors regulating the β -adrenergic agonistic response, we used different full and partial agonists including prenalterol. The extensive work by Kenakin and co-workers (Kenakin & Beek, 1980; Kenakin, 1982) showed that prenalterol is a partial agonist with a varying degree of ISA in different tissues, depending on the stimulus-response relationship (the so-called 'tissue factor') in each tissue.

A positive correlation was also found between the potency of isoprenaline and the efficacy of prenalterol in various tissues (Kenakin & Beek, 1980). This was verified and extended by Mattsson *et al.* (1983) who showed that a large number of spare receptors characterizes tissues sensitive to isoprenaline stimulation. This can be expressed as a high occupancy/potency ratio (K_d/EC_{50}) and a positive correlation was found between ISA for prenalterol and the K_d/EC_{50} ratio for isoprenaline in various tissues (Mattsson *et al.*, 1983).

In the present study it was found that the sensitivity to isoprenaline was low in normal adipose cells (EC_{50} 0.1 μM). This suggests that the number of spare receptors is low in rat adipocytes which is also in accordance with the finding that prenalterol is a weak agonist in normal adipose cells.

Treating the animals with pertussis toxin inhibited the G_i -protein activity as well as the ADP-ribosylation *in vitro* (Fig. 1). The functional inactivation of G_i -protein was also obvious from the accentuated stimulatory effect of GTP on adenylate cyclase activity (Fig. 5).

The inactivation of G_i -protein profoundly influenced the cellular sensitivity and responsiveness to prenalterol as well as the other partial β -adrenergic agonists. However, the responsiveness to a full agonist like isoprenaline was not altered, only the sensitivity was markedly increased. The reasons for these effects are mainly attributable to (1) the bimodal regulation of the adenylate cyclase, (2) the non-linear relationship between cAMP levels and biological effect and (3) a priming effect of low cAMP levels on PKA. These points are further elaborated on below.

(1) The isoprenaline-stimulated adenylate cyclase activity was markedly increased after pertussis toxin treatment, whereas the EC_{50} was essentially unchanged. The sensitivity and responsiveness to prenalterol could not be estimated since no consistent measurable activation of the adenylate cyclase was found. Since lipolysis clearly is maximally stimulated at submaximal cyclase activation, the increased activity of this enzyme would not influence the lipolytic responsiveness to a full agonist. In accordance with this, pertussis toxin treatment did not increase the lipolytic response to a high isoprenaline concentration. Nevertheless, the increased cyclase activity will augment the cAMP response at all agonist concentrations and thereby increase the cellular sensitivity to isoprenaline. We also conclude that, contrary to the conditions with a full agonist, when lipolysis is stimulated by partial agonists like prenalterol, terbutaline and β -

Fig. 7. Effects of cAMP analogues on lipolysis

Results are means of two to three experiments. (a) The effect of *N*⁶-monobutyryl cAMP on adipocytes from control (○) and pertussis-toxin-treated (●) rats. (b, c) The effect of 0.1 μ M-prenalterol (■) on the lipolytic effect of 8-bromo-cAMP (b) and *N*⁶-monobutyryl-cAMP (c) in adipocytes from control rats [◆, control (no prenalterol)]. (d) The effect of 8-bromo-cAMP (1 mM) (●) on the lipolytic effect of prenalterol in adipocytes from control rats [○, control (no 8-bromo-cAMP)]. B, basal.

adrenergic antagonists with ISA, activation of the adenylate cyclase is the rate-limiting step for the biological effect. This concept is also in agreement with G_i-protein being an important 'tissue factor' modulating the agonistic response to partial agonists.

(2) A small increase in the intracellular cAMP level is associated with a marked increase in biological response. In spite of the increased responsiveness of the adenylate cyclase to β -adrenergic stimulation and the marked concomitant increase in glycerol release in pertussis-toxin-treated cells, prenalterol only elicited a small increase in the cellular cAMP levels. However, the relationship between cellular cAMP levels and glycerol release clearly shows that the lipolytic process is highly sensitive to low intracellular cAMP concentrations but less sensitive to high concentrations. Also, in cardiac muscle prenalterol elicits a near-maximal physiological response with only a small increase in cAMP level (Hedberg *et al.*, 1982). It cannot be excluded of course that cAMP exists in different cell compartments where only one fraction is functional in activating PKA (Kuo & De Renzo 1969; Fain *et al.*, 1972). In this context, the subcellular localization of PKA, determined by binding to different cytoskeletal proteins, may be of importance (Scott *et al.*, 1990).

(3) Basal lipolysis was elevated in cells from pertussis-toxin-treated rats, and this was probably due to slightly higher basal cAMP levels, although the sensitivity of the cAMP assay was not sufficiently high to allow a direct demonstration of this. These cells were also more sensitive to the effect of exogenous cAMP addition measured as a shift in the dose-response curve for the non-degradable cAMP analogue, *N*⁶-monobutyryl-cAMP (Fig. 7a). Likewise, in control cells the sensitivity to both 8-bromo-cAMP and *N*⁶-monobutyryl-cAMP was increased by the addition

of a submaximal concentration of prenalterol (Figs. 7b and 7c). Furthermore, addition of a low concentration of 8-bromo-cAMP increased the effect of prenalterol (Fig. 7d). In all these experimental conditions, a small increase in basal cAMP level leads to an augmentation of the response to a further increase in cAMP, caused by either prenalterol or a cAMP analogue. This amplification of the response is not only additive but synergistic. From the extensive work by Corbin and collaborators (Rannels & Corbin, 1981; Robinson-Steiner & Corbin, 1983; Beebe *et al.*, 1984) it is known that PKA is an important step in sensitivity amplification. They have established a positive co-operativity in both cyclic nucleotide binding to the two binding sites of the regulatory subunit of PKA and PKA activation, as well as in stimulation of lipolysis by cAMP analogues.

Our data show that this is valid also for the combined effect of endogenous cAMP and a cAMP analogue. Both a site-1-selective (8-bromo-cAMP) and a site-2-selective (*N*⁶-monobutyryl-cAMP) analogue acts synergistically with endogenous cAMP in stimulating lipolysis (Figs. 7a-7d). The mechanism for this synergism must be beyond the adenylate cyclase in the lipolytic cascade reaction, as it is seen with cAMP analogues. The enhancing effect of both non-degradable and degradable analogues argues against the importance of phosphodiesterase for this effect. Thus PKA is the most probable level for the signal amplification seen.

Our results show that this co-operativity on PKA is also valid for physiological conditions when lipolysis is stimulated by a β -adrenergic agonist. At submaximal agonist concentrations causing small increases in intracellular cAMP, the co-operative effect of cAMP on the two binding sites of PKA elicits a physiological response that is unproportionally large in relation to the small

increase in cAMP levels. This is most likely an important reason for the very marked effect of pertussis toxin treatment on the lipolytic effect of prenalterol at very low cAMP concentrations.

The fact that prenalterol truly exerted its effect through the β -adrenergic adenylate cyclase complex and a concomitant cAMP elevation is supported by several findings. (1) Prenalterol dose-dependently inhibited isoprenaline-stimulated adenylate cyclase activity. (2) Prenalterol-stimulated lipolysis in control cells was increased by ADA and this effect was accentuated by both IBMX and OPC 3911. ADA degrades adenosine accumulating in the incubation medium, thereby eliminating its inhibitory effect on adenylate cyclase via stimulation of G_i -protein. IBMX inhibits both phosphodiesterase activity and adenosine binding (Londos *et al.*, 1978; Yamamoto *et al.*, 1984), whereas OPC 3911 is a specific inhibitor of the cGMP-inhibited low- K_m phosphodiesterase (Hidaka *et al.*, 1979; Degerman *et al.*, 1987). Both these agents thus increase the cellular cAMP levels in the presence of a β -adrenergic agonist. The more pronounced effect of pertussis toxin compared with ADA may be explained by the fact that G_i -protein is not only modulated by adenosine but also by other endogenous factors such as the prostaglandins.

In conclusion, only partial activation of the adenylate cyclase and a concomitant small increase in the cAMP level is required for a near-maximal biological response. This is due to the non-linear relationship between cAMP and the physiological response with a high sensitivity for low cAMP concentrations. Furthermore, low cAMP levels seem to exert a priming effect on the PKA increasing its sensitivity to a further stimulation. A priming effect of cAMP may have profound physiological implications for the interaction between the sympathetic nervous system and/or various hormones exerting their effects by increasing the intracellular cAMP levels.

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