NICOTINIC ACID INHIBITS ADIPOCYTE ADENYLATE CYCLASE IN A HORMONE–LIKE MANNER

K. AKTORIES, K. H. JAKOBS and G. SCHULTZ
Pharmakologisches Institut, Universität Heidelberg Im Neuenheimer Feld 366, D-6900 Heidelberg, FRG

Received 8 April 1980

1. Introduction

The cellular cyclic AMP level is an important factor controlling the rate of lipolysis in fat cells [1,2]. Various hormones such as ACTH, glucagon and β-adrenergic agonists increase the rate of lipolysis by increasing the intracellular concentration of cyclic AMP. On the other hand, the antilipolytic effects of several hormonal factors appear to involve a decreased level of cyclic AMP. Whereas insulin has been shown to stimulate the activity of the low K_m form of cyclic AMP phosphodiesterase [3], the lowering effects of adenosine, α-adrenergic agonists and some prostaglandins on adipocyte cyclic AMP levels appear to be due to receptor-mediated inhibitions of adenylate cyclase [4–6]. Besides these hormonal factors, nicotinic acid acts as a powerful antilipolytic drug, and it has been shown to decrease cyclic AMP levels in intact adipocytes of several species [1,2]. We present data indicating that nicotinic acid can inhibit adenylate cyclase in rat and hamster fat cell ghosts under conditions identical with those required for inhibition of the enzyme by hormonal factors.

2. Materials and methods

Materials and methods used were essentially as in [7]. Male golden hamsters (60–100 g) and rats (120–160 g) were fed ad libitum. Preparation of fat cells and their ghosts was as in [8]. Membrane preparations from human platelets and rabbit myocardium were obtained as in [9,10].

Adenylate cyclase activity was determined as in [7]. The assay medium contained 20 μM [α-^{32}P]ATP, 10 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM cyclic AMP, 5 mM creatine phosphate, 0.4 mg creatine kinase/ml and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine–HCl buffer (pH 7.4). Incubations were carried out in 100 μl total vol. at 25°C for 10 min. Reactions were initiated by the addition of membrane preparations (5–20 μg protein) to the prewarmed reaction mixture. For sodium-free conditions, creatine phosphate was used as its di-ammonium salt, prepared from the di-sodium salt by anion-exchange chromatography on QAE–Sephadex A-25. Assays were performed in triplicate in repeated experiments. Protein was determined according to [11], using bovine serum albumin as standard.

3. Results and discussion

Prostaglandin E_1 and adrenaline, by its α-adrenergic component, inhibit adenylate cyclase in ghosts of hamster adipocytes and effective inhibitory coupling requires the presence of both GTP and Na^+ [5]. In the same tissue, comparable results were obtained with nicotinic acid (Table 1). In the absence of GTP and NaCl or in the presence of NaCl (100 mM) alone, nicotinic acid (30 μM) had no effect, and the agent caused only slight inhibition in the presence of GTP (1 μM) alone. In the presence of both GTP and NaCl inhibition by nicotinic acid was largely pronounced. This effect, similar to inhibition by the hormonal factors, was immediate in onset and was seen with the basal and hormonally stimulated forms of the enzyme but was not demonstrable after activation by the stable GTP analogue, guanylyl 5′-imidodiphosphate [6]. The findings that nicotinic acid inhib-

Table 1
Inhibition of hamster adipocyte adenylate cyclase by nicotinic acid, prostaglandin E_1 and adrenaline

Additions	Control	Nicotinic acid (30 μM)	Prostaglandin E_1 (1 μM)	1-Adrenaline (300 μM)
	(pmol cAMP . mg protein^{-1} . min^{-1})			
None	279 ± 10	272 ± 15	281 ± 25	287 ± 7
NaCl (100 mM)	213 ± 3	206 ± 2	210 ± 8	208 ± 5
GTP (1 μM)	48.2 ± 0.8	39.3 ± 1.5	51.9 ± 0.1	52.6 ± 1.1
NaCl + GTP	95.1 ± 2.8	56.6 ± 0.3	55.9 ± 0.4	64.3 ± 0.6

Indicated are means ± SD from a typical experiment. The effect of l-adrenaline was studied with 30 μM d,l-propranolol, which by itself had no effect

ited hamster adipocyte adenylate cyclase in a hormone-like manner initiated studies on the problem of whether nicotinic acid causes similar inhibition of the enzyme in adipocytes from a different species and in two different cellular systems.

In rat fat cell ghosts, neither in the presence of GTP (1 μM) alone nor of NaCl (100 mM) alone, nicotinic acid (up to 100 μM) significantly affected adenylate cyclase activity (fig.1). However, when added in the presence of both GTP and NaCl, nicotinic acid inhibited the rat fat cell enzyme to a similar extent and with a similar concentration-dependence as observed in hamster adipocytes [6]. Half-maximal inhibition occurred at ~0.5 μM, and maximal inhibition by 40–60% was seen at ~10 μM nicotinic acid.

Depicted in fig.2 is the influence of GTP on the inhibitory effect of nicotinic acid (30 μM) on rat adipocyte adenylate cyclase activity in the presence of 120 mM NaCl. As reported for the hamster and rat adipocyte enzyme [5,12], GTP decreased the activity in a concentration-dependent manner. In the presence of 120 mM NaCl, the GTP-induced reduction of activity was half-maximal at ~1 μM, and maximal reduction by ~50% occurred at 10–30 μM GTP. The adenylate cyclase inhibition by nicotinic acid depended on the presence of GTP. Maximal inhibition by the antilipolytic compound was seen at 1 μM GTP; the effect was half-maximal at ~0.2 μM GTP. At > 1 μM GTP, the inhibitory effect slightly decreased.

The influence of Na^+, added as NaCl, on the nicotinic acid-induced inhibition of rat adipocyte adenylate cyclase is presented in fig.3. NaCl increased the enzyme activity suppressed by GTP (1 μM) maximally 4-fold at ~300 mM; this effect was half-maximal at ~100 mM NaCl. Nicotinic acid (30 μM) specifically reduced this NaCl-induced increase in activity, whereas the agent had only a small effect in the absence of NaCl. Maximal inhibition by nicotinic acid was seen between 70–150 mM NaCl, and half-maximal inhibition occurred at ~30 mM. These findings on the GTP- and Na^+-requirements are very similar to those obtained in hamster adipocytes for inhibition by nicotinic acid, prostaglandin E_1 and the

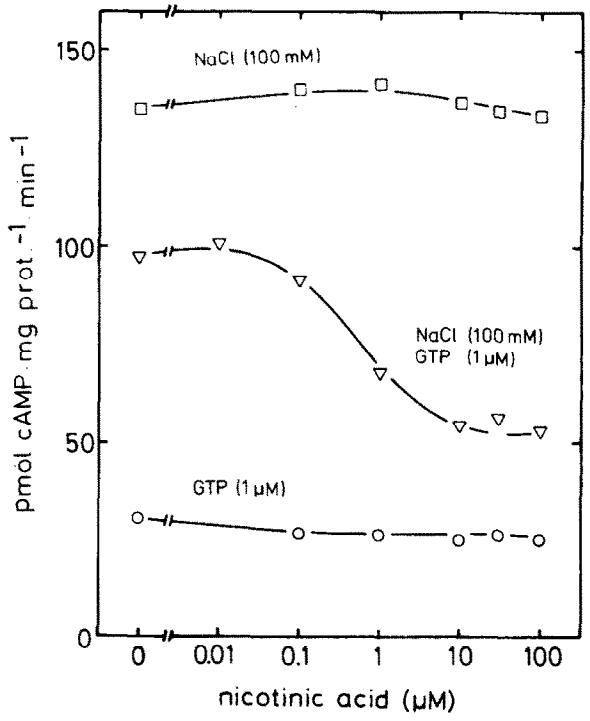

Fig.1. Effect of nicotinic acid on rat adipocyte adenylate cyclase activity. The effect of nicotinic acid added at the concentrations indicated on the abscissa was studied in the presence of 1 μM GTP (o), 100 mM NaCl (□) or GTP plus NaCl (▽).

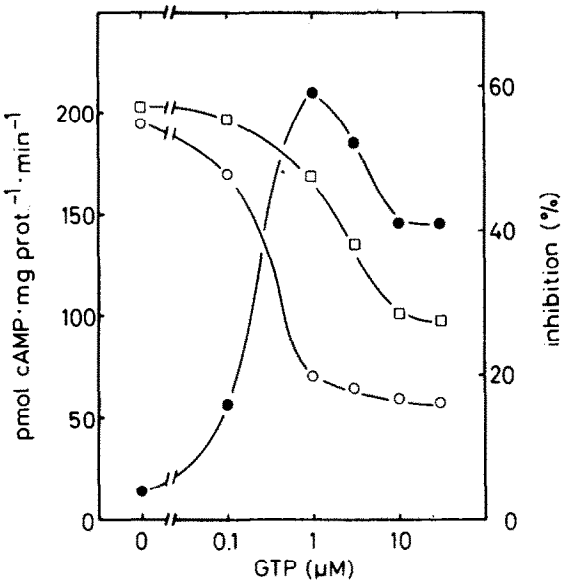

Fig.2. Influence of GTP on the effect of nicotinic acid on rat adipocyte adenylate cyclase activity. Enzyme activity (left ordinate) was determined at the GTP concentrations indicated on the abscissa in the absence (□) or presence (o) of 30 μM nicotinic acid. NaCl (120 mM) was present under each condition. (•) Inhibition (%, right ordinate) by nicotinic acid.

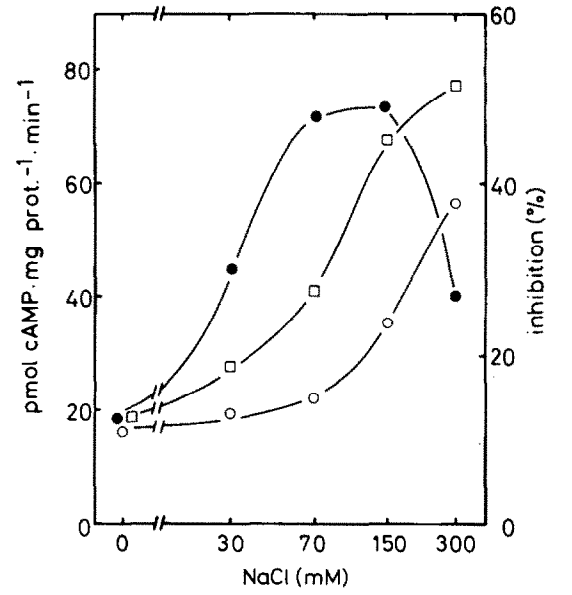

Fig.3. Influence of NaCl on the effect of nicotinic acid on rat adipocyte adenylate cyclase activity. Enzyme activity (left ordinate) was determined at the NaCl concentrations indicated on the abscissa in the absence (□) or presence (o) of 30 μM nicotinic acid. GTP (1 μM) was present under each condition. (•) Inhibition (%, right ordinate) by nicotinic acid.

α-adrenergic component of adrenaline [5,6]. Similar to hormone-induced inhibitions of adenylate cyclases, the effect of nicotinic acid on the rat adipocyte enzyme could be reversed by washing of the membrane preparations (not shown). An indirect effect of nicotinic acid on adenylate cyclase, involving an increased formation of a prostaglandin of the E type, is not likely as indomethacin (100 μM) or acetylsalicyclic acid (100 μM) did not affect nicotinic acid-induced inhibition of adenylate cyclase (not shown).

As an interaction of nicotinic acid with specific receptors has not been shown, we have studied a possible tissue-non-specific interaction of this compound with the coupling system responsible for transducing inhibitory signals from hormone receptors to the adenylate cyclase. In human platelets and rabbit myocardium, α-adrenergic and cholinergic agonists, respectively, exhibit a GTP-dependent inhibition of the adenylate cyclase [9,10]. However, under the same conditions when these hormonal factors were inhibitory, nicotinic acid (30 μM) had no effect on the enzyme of these tissues (table 2).

Table 2
Effect of nicotinic acid on adenylate cyclase of human platelets and rabbit myocardium

		(pmol cAMP . mg protein^{-1} . min^{-1})
Human platelets		
Control		57.9 ± 4.2
Adrenaline	30 μM	40.3 ± 0.7
	300 μM	37.1 ± 1.1
Nicotinic acid	30 μM	57.5 ± 4.8
	300 μM	52.4 ± 1.1
Rabbit myocardium		
Control		31.7 ± 1.4
Carbachol	100 μM	19.3 ± 1.1
Nicotinic acid	30 μM	29.8 ± 1.1

Adenylate cyclase assay conditions were essentially as in section 2. [α-^{32}P]ATP, GTP and NaCl were 50 μM, 30 μM and 80 mM, respectively. Ethyleneglycol-bis(β-aminoethylether)-*N,N'* tetraacetic acid (EGTA, 0.1 mM) was present under all conditions. The myocardial activity was determined in the presence of 1 mM dithiothreitol (DTT) and 10 mM creatine phosphate. Incubations were carried out for 20 min at 37°C with 16 μg platelet membrane protein and for 10 min at 25°C with 85 μg myocardial protein. The effect of l-adrenaline was studied with 30 μM d,l-propanolol, which by itself had no effect on the enzyme activity. Numbers indicate means ± SD of the adenylate cyclase activities from a typical experiment

These data presented indicate that inhibition of rat and hamster adipocyte adenylate cyclase resembles that seen with various hormonal factors in different cell types [4–6,9,10,13,14]. Common characteristics of the inhibitions by nicotinic acid as well as by hormonal factors are the velocity, magnitude and reversibility of the effects and the simultaneous requirements for GTP and Na^+. As an unspecific effect of nicotinic acid (beyond the hormone receptor level) could not be detected, the data suggest that the effect of nicotinic acid on adipocyte adenylate cyclase involves a specific membrane-bound receptor. Further studies are required to demonstrate the occurrence of the postulated receptor in binding studies and to elucidate its physiological ligand.

Acknowledgements

The authors are indebted to Miss Heidrun Vogt and to Miss Gabriele Gabel for excellent technical assistance. These studies were supported by the Deutsche Forschungsgemeinschaft.

References

[1] Fain, J. N. (1973) Pharmacol. Rev. 25, 67–118.
[2] Burns, T. W., Langley, P. E. and Robison, G. A. (1972) Adv. Cyclic Nucl. Res. 1, 63–84.
[3] Loten, E. G. and Sneyd, J. G. T. (1970) Biochem. J.. 120, 187-193.
[4] Londos, C., Cooper, D. M. F., Schlegel, W. and Rodbell, M. (1978) Proc. Natl. Acad. Sci. USA 75, 5362-5366.
[5] Aktories, K., Schultz, G. and Jakobs, K. H. (1979) FEBS Lett. 107, 100-104.
[6] Aktories, K., Schultz, G. and Jakobs K. H. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. in press.
[7] Jakobs, K. H., Saur, W. and Schultz, G. (1976) J. Cyclic Nucl. Res. 2, 381-392.
[8] Hittelman, K. H., Wu, C. F. and Butcher, R. W. (1973) Biochim. Biophys. Acta 304, 188-196.
[9] Jakobs, K. H., Saur, W. and Schutz, G. (1978) FEBS Lett. 85, 167-170.
[10] Jakobs, K. H., Aktories, K. and Schultz, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 113-119.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 248, 6239-6245.
[12] Harwood, J. P., Löw, H. and Rodbell, M. (1973) J. Biol. Chem. 248, 6239-6245.
[13] Sabol, S. L. and Nirenberg, M. (1979) J. Biol. Chem. 254, 1913-1920.
[14] Blume, A. J., Lichtshtein, D. and Boone, G. (1979) Proc. Natl. Acad. Sci. USA 76, 5626–5630.