Klin. Wschr. 55, 625–628 (1977)

Klinische Wochenschrift

Originalien

β-Adrenergic Receptor Coupled-Adenylate Cyclase of Human Fat Cell Ghosts*

H. Kather, B. Vogt and B. Simon

Klinisches Institut für Herzinfarktforschung an der Medizinischen Universitätsklinik Heidelberg

Das an adrenerge β-Rezeptoren gekoppelte Adenylat Cyclase System in menschlichen Fettzellen

Zusammenfassung. Die Wirkungen beta-adrenerger Substanzen wie Isoproterenol, Adrenalin und Noradrenalin wurden entweder allein oder zusammen mit Propranolol, einem Beta-Blocker, auf das Adenylat-Cyclase System aus menschlichem Unterhautfettgewebe untersucht. Einen Katecholamin-Sensitivität des menschlichen Enzymsystems ließ sich ohne Zusatz von künstlichen Kofaktoren nachweisen. Maximal-Konzentrationen von Isoproterenol, Adrenalin und Nor-Adrenalin führten zu einer 2–6,5fachen Zunahme der Enzym-Aktivität. Isoproterenol stimulierte deutlich stärker als die beiden Hormome. Propranolol führte zu einer kompetitiven Hemmung des stimulierenden Effektes der beta-adrenergen Agonisten. Es wird gefolgert, daß die in-vitro Messung der Adenylat-Cyclase Aktivität im menschlichem Fettgewebe eine einfache Möglichkeit zur Testung von Substanzen mit adrenerger Wirkung darstellt.

Schlüsselwörter: Adenylat-Cyclase – Katecholamine – β-adrenerge Rezeptoren – Menschliches Fettgewebe.

Summary. The effects of beta-adrenergic agonists such as isoproterenol, norepinephrine and epinephrine upon the adenylate cyclase activity of human fat cell ghosts were tested, each alone and in combination with the beta-blocking agent propranolol. Saturating concentrations of these agents showed a 2–6.5-fold increase of enzyme activity without addition of any artificial cofactors. Isoproterenol was more potent in stimulating the enzyme system than epinephrine and nor-epinephrine. Propranolol caused a dose-dependent rightward shift of the log-dose response curve of these beta-adrenergic agonists. The assay of human fat cell adenylate cyclase in vitro may provide a simple and convenient assay system for the screening of beta-adrenergic drugs of potential therapeutic importance.

Key words: Adenylate Cyclase – Catecholamines – β-adrenergic receptor – Human adipose tissue.

* Herrn Prof. Dr. Dr. h.c. G. Schettler zum 60. Geburtstag gewidmet

Introduction

The fat cell adenylate cyclase was shown to play an important role in hormone-stimulated lipolysis. The *rat* fat cell enzyme reacts with various hormones such as ACTH, TSH and secretin (Birnbaumer et al., 1969; Birnbaumer and Rodbell, 1969). Attempts to demonstrate a hormone-sensitive adenylate cyclase in broken cell preparation from *human* fat cells led to conflicting results. Burns et al. (1972) and Cooper et al. (1975) observed only low and non-reproducible sensitivity towards epinephrine unless phentolamine or 5′-Guanylyl-imido-diphosphate were included in the assay medium.

These findings are in contrast to the results of others (Poupon, 1975; Kather and Geiger, 1975; Kather et al., 1976; Kather et al., 1976; Geiger et al., 1976; Simon-Crisan et al., 1976; Kather and Geiger, 1976) who observed a significant response towards epinephrine without addition of artificial cofactors.

To provide further data in support of the concept that the action of catecholamines is mediated via activation of the human fat cell adenylate cyclase, we compared the effects of different β-adrenergic agonists such as isoproterenol, nor-epinephrine and epinephrine in the absence and presence of the β-blocking agent propranolol. It is shown that human fat cell ghosts contain an adenylate cyclase system coupled to a β-adrenergic receptor.

Methods

Source of Biopsies

Biopsies of subcutaneous adipose tissue were obtained from 10 patients undergoing surgical treatment. No attempt was made to select the patients on the basis of age, sex, weight or disease, except that cachectic persons were excluded. The patients were operated after an overnight fast. Anesthesia was induced with a short acting barbiturate and maintained with halothane, nitrous oxide and oxygen. The biopsies were usually obtained after the skin incision at the start of the operation.

Preparation of Fat Cell Ghosts

Adipose tissues were cut into 20–25 mg fragments and fat cells were isolated essentially according to Rodbell (1972), except that higher concentrations of collagenase (3 mg/ml) and Tris-HCl buffer (0.025 M), pH 7.4, instead of Krebs-Ringer-bicarbonate buffer were used.

Fat cell ghosts representing a plasma membrane-rich fraction isolated from hypotonic lysates of free fat cells were prepared according to Rodbell (1972). The lysing medium contained 0.05 M $MgCl_2$, 0.05 M ATP, 0.05 M $CaCL_2$, 0.1 M $KHCO_3$ and Tris-HCl (0.01 M), pH 7.6. The ghosts were suspended in 0.1 mM $KHCO_3$ in a final concentration of 0.25 to 2.5 mg protein per ml.

Adenylate Cyclase Assay

The adenylate cyclase activity of fat cell ghosts was determined according to Salomon et al. (1974). The incubation mixture contained 25 mM Tris-HCl, pH 8.5, 5 mM $MgCl_2$, 20 mM creatine phosphate, 100 U/ml creatine phosphokinase, 1 mM cyclic AMP and 1 mM (α-^{32}P)-ATP (40–50 cpm/pmole). L(−)-epinephrine, L(−)-norepinephrine and L(−)-isoproterenol were dissolved in 0.01 N HCl and neutralized immediately before use by addition of Tris.

The reaction was initiated by the addition of 20 µl of suspended ghosts (1 µg–20 µg of ghost protein) and terminated by addition of 0.1 ml of stopping solution composed of 2% lauryl sulfate, 1.4 mM cAMP and 40 mM ATP. To monitor column losses approximately 30.000 cpm of ^{3}H cAMP in a volume of 0.1 ml were added immediately after stopping. Cyclic (^{32}P)-AMP was purified by column chromatography using Dowex AG 50 W-X4 and neutral alumina.

Data are given as nmol of cAMP formed per mg protein per 15 min. Statistical analysis was performed by the Wilcoxon-test for paired samples.

The protein content of the samples was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

Materials

(α-^{32}P) ATP (2–6 counts/mmole) and cyclic (^{3}H) AMP (27 counts/mmole) were purchased from Radiochemical Centre, Amersham, Bucks, U.K.

L(−)-epinephrine and L(−)-norepinephrine were obtained from Merck AG, Darmstadt, L(−)-isoproterenol and (−)-propranolol from Boehringer-Ingelheim, F.R.G.

All other chemicals and reagents were of the highest grade obtainable commercially.

Results

Basal activity of the human fat cell adenylate cyclase averaged 1.4 ± 0.2 nmol cAMP formed per mg protein per 15 min in 10 separate experiments (data not shown).

Depicted in Figure 1 are the typical dose-response curves of the three beta-adrenergic agonists isoproterenol, epinephrine and norepinephrine. It can be seen that the order of potency of these agents upon enzyme stimulation is isoproterenol > epinephrine > norepinephrine. In these experiments the K_D-values, i.e. the concentration of each agent causing half maximal enzyme stimulation, were shown to be for isoproterenol 1.0 µM, for epinephrine 10 µM and for norepinephrine 30 µM.

Saturating concentrations of norepinephrine (5×10^{-4} M) used a significant increase of enzyme activity by about 200–300%. Epinephrine (5×10^{-4} M) was significantly more potent in stimulating the human fat cell enzyme than norepinephrine (about 450% stimulation), ($p \leqq 0.05$). Isoproterenol (5×10^{-5} M) increased enzyme activity by about 550%.

The affinity of the antagonist propranolol for the adenylate cyclase-coupled beta-adrenergic receptors was assessed by quantitating the ability of this beta-blocking agent to cause a parallel rightward shift of the isoproterenol dose-response curve. Propranolol, when used in concentrations of 5×10^{-8} M and

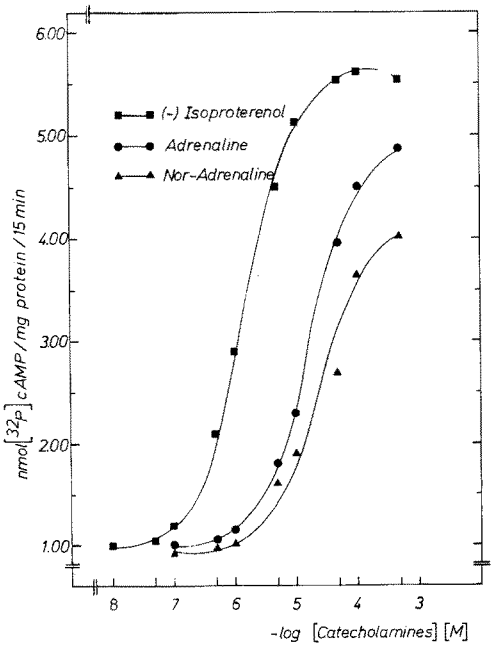

Fig. 1. Stimulation of human fat cell adenylate cyclase by beta-adrenergic agonists. The results are the mean values of three experiments determined in duplicate

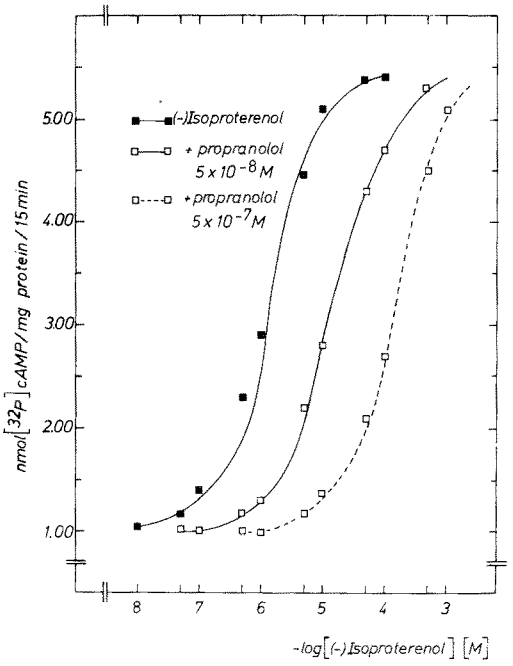

Fig. 2. Antagonism of isoproterenol stimulation of human fat cell adenylate cyclase by two different concentrations of propranolol. Results are the mean values of three experiments determined in duplicate

5×10^{-7} M, inhibited the effect of isoproterenol in a dose-dependent fashion. Similar effects of propranolol were obtained in the presence of epinephrine and norepinephrine (data not shown).

Propranolol (from 1×10^{-8} M upt to 1×10^{-4} M) was also tested as agonist and had no intrinsic activity on the adenylate cyclase system of human fat cell ghosts in the absence of hormones.

Discussion

Lipolytic studies carried out with adipose tissue of various species suggest that the effects of catecholamines are mediated via interaction with beta-adrenergic receptors (Rudman and Di-Girolamo, 1967; Himms-Hagen, 1970). Beta-adrenergic receptors have shown a characteristic higher affinity for isoproterenol than for epinephrine and norepinephrine (see Fain, 1973). Antagonists such as propranolol have also high affinity for the beta-receptors (Fain, 1973).

In two recent communications artificial cofactors such as the guanine-nucleotide GMP-P(NH)P (guanylyl-imidodiphosphate) or phentolamine (an alpha blocking agent) have been reported to be essential for expression of catecholamine-sensitivity of the human fat cell adenylate cyclase system (Cooper et al., 1975; Burns et al., 1972). Both compounds are not present in the organism. To provide support for the physiological role of the *human* fat cell adenylate cyclase system in catecholamine action, it was attempted to demonstrate hormone responsiveness under more physiological conditions. In this study we have demonstrated that the human fat cell adenylate cyclase system reacts to beta-adrenergic agonists without addition of artificial cofactors. Catecholamine-induced activation was competitively inhibited by the beta-blocking agent propranolol. This finding coupled with the potency of isoproterenol indicates that the receptors are of the beta-adrenergic type. These results support the contention that the beta-adrenergic receptors coupled to the adenylate cyclase system are physiologically relevant in human adipocytes.

Large variations has been observed between species in both qualitative and quantitative aspects of the lipolytic response of adipose tissue to adrenergic agents (Rudman and Di-Girolamo, 1967). In vitro experiments with human tissue preparation are therefore of great value in estimating the potential therapeutic usefulness of new beta-adrenergic substances. Subcutaneous adipose tissue is readily available in sufficient amount by open biopsy from surgical patients or by needle biopsy from volunteers. Thus, the adenylate cyclase system of human fat cell ghosts appears to be a simple and convenient assay system for screening new, potentially specific beta-adrenergic drugs.

References

Birnbaumer, L., Pohl, L.S., Rodbell, M.: Adenyl cyclase in fat cells. I. Properties and the effects of adrenocorticotropin and fluoride. J. biol. Chem. **244**, 3468–3476 (1969)

Birnbaumer, L., Rodbell, M.: Adenyl cyclase in fat cells. II. Hormone receptors. J. biol. Chem. **244**, 3477–3482 (1969)

Burns, T.W., Langley, P.E., Robison, G.A.: Studies on the role of cyclic AMP in human lipolysis. Adv. Cyc. Nucl. Res. **1**, 63–85 (1972)

Cooper, B., Partilla, J.S., Gregerman, R.I.: Adenylate cyclase of human fat cells; expression of epinephrine-sensitive activation revealed by 5′-guanylyl-imidodiphosphate. J. clin. Invest. **56**, 1350–1353 (1975)

Fain, J.N.: Biochemical aspects of drug and hormone action on adipose tissue. Pharmacol. Rev. **25**, 67–118 (1973)

Geiger, M., Simon-Crisan, G., Simon, B., Kather, H.: Untersuchungen über die Adenylzyklase aus menschlichem Fettgewebe. Verh. Ges. Inn. Med. (82. Tagung) 1976

Himms-Hagen, J.: Adrenergic receptors for metabolic responses in adipose tissue. Fed. Proc. Fed. Amer. Soc. exp. Biol. **29**, 1388–1401 (1970)

Kather, H., Geiger, M.: Hormone-sensitive (and NaF) adenylate cyclase from human adipocytes. Xth Acta Endocrin. (Kbh.) Suppl. 275 (1975)

Kather, H., Simon-Crisan, G., Simon, B.: Inhibition of human fat cell adenylate cyclase by clofibrate. Horm. Metab. Res. **8**, 246–247 (1976)

Kather, H., Simon-Crisan, G., Vogt, B., Simon, B.: Effects of clofibrate on the human fat cell adenylate cyclase system. Horm. Metab. Res., in press (1977)

Kather, H., Geiger, M.: Epinephrine-sensitive adenylate cyclase of human fat cell ghosts Properties, hormone-sensitivity and the effects of guanine nucleotides. Europ. J. clin. Invest. (in press) (1977)

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193**, 265–275 (1951)

Poupon, R.: Activity of human adenylate cyclase from human fat cell membranes. Biomedicine **23**, 438–442 (1975)

Rodbell, M.: Methods of cyclic nucleotide research (M. Chasin, ed.), pp. 101–124. In. New York: Marcel Bekker 1972

Rudman, D., Di-Girolamo, M.: Comparative studies on the physiology of adipose tissue. Adv. Lip. Res. **5**, 35–117 (1967)

Salomon, Y., Londos, C., Rodbell, M.: A highly sensitive adenylate cyclase assay. Anal. Biochem. **58**, 541–548 (1974)

Simon-Crisan, G., Vogt, B., Simon, B., Kather, H.: Inhibition of human fat cell adenylate cyclase by clofibrate. 7th Intern. Symp. Clin. Enzymology, Venedig, 1976, Proc. Vol. (in press)

Dr. H. Kather
PD Dr. B. Simon
Klinisches Institut für Herzinfarktforschung
Med. Univ.-Klinik
Bergheimerstr. 58
D-6900 Heidelberg
Federal Republic of Germany