Effect of pertussis toxin on α_2-adrenoceptors: decreased formation of the high-affinity state for agonists

J. Adolfo García-Sáinz*, José Luis Boyer*+, Thomas Michel, Diane Sawyer, Gary L. Stiles, Henrik Dohlman and Robert J. Lefkowitz

Departamento de Bioenergética, Centro de Investigaciones en Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-600, D.F. 04510 Mexico, +Departamento de Endocrinología, Instituto Nacional de Cardiología Ignacio Chávez, Mexico and *Department of Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710, USA*

Received 2 April 1984

Administration of pertussis toxin to hamsters abolishes the α_2-adrenergic inhibition of adenylate cyclase in their adipocytes. The toxin did not modify the number of adipocyte α_2-adrenoceptors or their affinity for antagonists. In contrast, the affinity for agonists was significantly diminished in adipocyte membranes obtained from pertussis toxin-treated hamsters as compared to the controls. This decrease in affinity was due to a significant diminution in the proportion of sites displaying the high-affinity state for agonists. It is concluded that pertussis toxin induces a shift in the proportion of sites in high- and low-affinity states for agonists towards the low-affinity conformation.

Pertussis toxin *α_2-Adrenoceptor* *Receptor affinity*

1. INTRODUCTION

In many cell types, pertussis toxin, one of the toxins produced by *Bordetella pertussis*, blocks the inhibition of adenylate cyclase mediated by hormones and neurotransmitters [1–9]. The effect of pertussis toxin seems to be due to the NAD-ribosylation of a protein (M_r 41000) which presumably is a subunit of Ni [3,4,10,11]. It has been reported that this toxin-catalyzed covalent modification of Ni is associated with blockage of the low-K_m GTPase activity linked to receptor-mediated inhibition of adenylate cyclase [13–15]. Furthermore, two recent reports indicate that the toxin decreases the affinity for agonists of receptors coupled inhibitorily to the cyclase [4,12].

Adipocytes are a model system well known for their exquisite sensitivity to agents that activate and inhibit adenylate cyclase. The α_2-adrenoceptors present in hamster adipocytes are coupled inhibitorily to the cyclase and have been carefully characterized by our group [16]. Furthermore, the effect of pertussis toxin on this model is well documented [5,6,9,14]. We present here the results of a detailed study of the effect of pertussis toxin on hamster adipocyte α_2-adrenoceptors.

2. MATERIALS AND METHODS

[^{3}H]Dihydroergocryptine ([^{3}H]DHE) (spec. act. 33 Ci/mmol) was obtained from New England Nuclear. Collagenase (type II) and bovine serum albumin (fraction V) were from Worthington and Armour, respectively. l-Isoproterenol, l-epinephrine, propranolol, ACTH, dithiothreitol and guanylyl-5-imidodiphosphate (Gpp(NH)p) were obtained from Sigma. Prazosin was a generous gift from Pfizer.

Male Golden Hamsters (80–90 g) fed ad libitum were used. Pertussis toxin, purified ≈1800-fold as in [6], was administered intraperitoneally to the hamsters 3 days before killing at a dose of 10 μg/100 g [6]. Control animals received the vehicle. Adipocytes were obtained as in [17]. In studies

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

of cyclic AMP accumulation, fat cells ($\approx 2 \times 10^5$ cells) were incubated for 10 min at 37°C with the agents indicated in 1 ml Krebs-Ringer phosphate buffer containing 100 μM theophylline and 0.5 μg adenosine deaminase [5,6]. Cyclic AMP was quantified as in [18]. Adipocyte membranes were prepared as in [16]. α_2-Adrenergic receptors were studied using [^{3}H]DHE as in [16] with the following modifications: (i) 10^{-7} M prazosin was included in all the experiments to block α_1-adrenoceptors present in the membranes [16]; (ii) the incubation volume was increased to 0.25 ml, and 1 mM dithiothreitol was added; (iii) temperature was decreased to 25°C; (iv) the incubation period was increased to 30 min; these modifications allowed us to obtain much more reproducible results in the agonist competition experiments. Specific binding to α_2-adrenergic receptors was 70–80% at the K_d. Analysis of the data was performed by previously described computer modelling techniques [19–21].

3. RESULTS AND DISCUSSION

In agreement with [5–9], it was observed that in cells from pertussis toxin-treated hamsters the ability of agents, such as ACTH or isoproterenol, to stimulate adenylate cyclase was magnified whereas the inhibitory action of α_2-adrenergic amines was blocked (table 1). To gain further insight into the mechanism of such blockade by the toxin, the α_2-adrenoceptors of the adipocyte were studied.

Binding saturation experiments using [^{3}H]DHE indicated that neither the number of α_2-adrenoceptors nor their affinity for the antagonist was affected by the toxin: B_{max} 500 ± 39 and 534 ± 29 fmol/mg protein and K_d 5.8 ± 0.8 and 4.7 ± 0.5 nM in membranes from adipocytes from control and pertussis-treated hamsters, respectively (means ± SE of 5–6 experiments using different membrane preparations).

Competition experiments, however, indicated that the affinity of the adipocyte α_2-adrenoceptors for agonists was markedly affected by the toxin. Fig.1 presents the competition curves of epinephrine with [^{3}H]DHE in the absence or presence of 0.1 mM Gpp(NH)p. In membranes from control adipocytes the epinephrine competition curve with [^{3}H]DHE yielded a shallow binding isotherm with a slope factor significantly less than 1 suggesting the presence of heterogeneous binding states. Computer modelling indicated the presence of two classes of binding states with high (α_{2H}) and low (α_{2L}) affinity for epinephrine. Addition of Gpp(NH)p caused the competition curve to shift to the right, consistent with a lower affinity of the α_2-adrenoceptors for epinephrine and to become steep with a slope factor not different from 1. In adipocyte membranes obtained from toxin-treated hamsters the competition curve of epinephrine still gave a slope factor less than 1; analysis of the data indicated the presence of α_2-adrenoceptors in both α_{2H} and α_{2L} affinity states. However, the proportion of sites in each state was significantly different in fat cell membranes from control as compared to toxin-treated hamsters (table 2). The competition curve in the presence of Gpp(NH)p gave a binding isotherm not significantly different from that

Table 1

Effect of pertussis toxin on the hormonal regulation of cyclic AMP levels in hamster adipocytes

Addition	Cyclic AMP (pmol/10^6 cells)	
	Control	Pertussis
Basal	74 ± 3	82 ± 9
ACTH (10 μg/ml)	3497 ± 199	9518 ± 297
ACTH (10 μg/ml) + 10^{-5} M propranolol + 10^{-5} M epinephrine	365 ± 37	9179 ± 594
Isoproterenol (10^{-6} M)	5258 ± 408	9150 ± 395
Epinephrine (10^{-5} M)	182 ± 15	9531 ± 252

Each value is the mean ± SE of 12–16 determinations

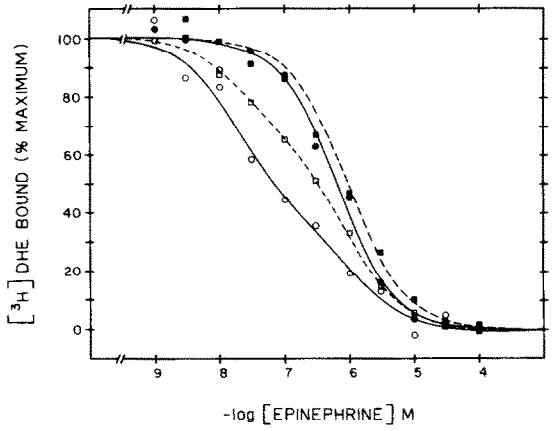

Fig.1. Competition curves of [^{3}H]DHE with epinephrine in the absence and presence of 0.1 mM Gpp(NH)p. (○) Control, (●) control + Gpp(NH)p, (□) pertussis, (■) pertussis + Gpp(NH)p. The data represent the mean of 6 experiments, each performed in duplicate. The concentration of [^{3}H]DHE was 3–6 nM. The data were normalized for graphic representation.

observed in the controls with the guanine nucleotide (fig.1, table 2). Our data indicate that pertussis toxin induces a shift to the low-affinity state for agonists of the α_2-adrenoceptors coupled inhibitorily to adenylate cyclase and are consistent with the results in [4] and [12].

Our data differ from those in [4,12] in that the shift to the low-affinity state for agonists induced by the toxin was not complete. This allowed us to demonstrate the transition from a majority of high- to low-affinity states by performing a detailed analysis of the data (table 2). The absence of complete blockade by the toxin under our conditions was previously shown by the inhibitory action of high concentrations of agonists on cyclic AMP levels and lipolysis [5,6]. We have observed a similar partial effect of pertussis toxin in renal α_2-adrenoceptors (submitted).

In conclusion, our studies indicate that pertussis toxin shifts the state of affinity of receptors coupled inhibitorily to adenylate cyclase towards the low-affinity state for agonists. Although obviously more direct studies are required to establish the molecular mechanism of such action of the toxin, the data suggest that the covalent modification of the 41-kDa protein alters the interactions of Ni with both the receptors and adenylate cyclase. The altered interaction of Ni with adenylate cyclase is shown by the blockade by pertussis toxin of the inhibitory action of GTP [8,14]. The relationship between these actions of the toxin and the enhanced response to stimulatory agents remains to be established. However, recent findings suggest the existence of a common subunit in Ni and Ns, which offers new possibilities of explaining the basic mechanism of the actions of the toxin on a molecular basis [10,11].

Table 2

Parameters derived from the computer modelling of competition curves of epinephrine with [^{3}H]DHE in adipocyte membranes from control and pertussis toxin-treated hamsters

Parameter	Control	Pertussis-treated	Control + Gpp(NH)p	Pertussis-treated + Gpp(NH)p
K_d α_{2H} (nM)	12.5 ± 7.6[a]	14.8 ± 7.1[a]		
K_d α_{2L} (nM)	720 ± 720[a]	580 ± 170[a]	400 ± 90	550 ± 60
α_{2H} (%)	64 ± 12[b]	38 ± 5[b]		
α_{2L} (%)	36 ± 10[b]	62 ± 5[b]	100	100
EC_{50} (nM)	78 ± 30[c]	330 ± 46[c]	700 ± 99	760 ± 130
Slope	0.60 ± 0.10[d]	0.71 ± 0.05[d]	1.1 ± 0.09	0.83 ± 0.09

[a] Two-state fit significantly better than one-state fit ($p < 0.04$)

[b] Proportion of receptors in high-affinity state was significantly different between control and pertussis-treated. Comparisons were made by comparing the goodness of fit by an F test as in [19–21]. Two-state fits were utilized only when this more complex model significantly improved the goodness of fit

[c] EC_{50} significantly different ($p < 0.002$)

[d] Slope different from 1 ($p < 0.04$)

ACKNOWLEDGEMENTS

R.J.L. is an Investigator of the Howard Hughes Medical Institute; T.M. is a student in the Medical Scientist Training Program (NIH grant GM07171). This research was partially supported by a Grant from the Fondo de Estudios e Investigaciones Ricardo J. Zevada. G.L.S. is supported by an NHLBI Clinical Investigator Award HL0127.

REFERENCES

[1] Hazeki, O. and Ui, M. (1981) J. Biol. Chem. 256, 2856–2862.
[2] Katada, T. and Ui, M. (1981) J. Biol. Chem. 256, 9310–9317.
[3] Katada, T. and Ui, M. (1982) Proc. Natl. Acad. Sci. USA 79, 3129–3133.
[4] Kurose, H., Katada, T., Amano, T. and Ui, M. (1983) J. Biol. Chem. 258, 4870–4875.
[5] García-Sáinz, J.A. (1981) FEBS Lett. 126, 306–309.
[6] Martínez-Olmedo, M.A. and García-Sáinz, J.A. (1983) Biochim. Biophys. Acta 760, 215–220.
[7] Moreno, F.J., Mills, I., García-Sáinz, J.A. and Fain, J.N. (1983) J. Biol. Chem. 258, 10938–10943.
[8] Fain, J.N., O'Donnell, C.J., Mills, I. and García-Sáinz, J.A. (1983) Biochem. Biophys. Res. Commun. 116, 651–656.
[9] Martínez-Olmedo, M.A. and García-Sáinz, J.A. (1984) Eur. J. Pharmacol., in press.
[10] Bokoch, G.M., Katada, T., Northup, J.K., Hewlett, E.L. and Gilman, A.G. (1983) J. Biol. Chem. 258, 2072–2075.
[11] Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R.D. and Manclark, C.R. (1983) Proc. Natl. Acad. Sci. USA 80, 4276–4280.
[12] Kuno, T., Shirakawa, O. and Tanaka, C. (1983) Biochem. Biophys. Res. Commun. 115, 325–330.
[13] Burns, D.L., Hewlett, E.L., Moss, J. and Vaughan, M. (1983) J. Biol. Chem. 258, 1435–1438.
[14] Aktories, K., Schultz, G. and Jakobs, K.H. (1983) FEBS Lett. 156, 88–92.
[15] Aktories, K., Schultz, G. and Jakobs, K.H. (1983) FEBS Lett. 158, 169–173.
[16] García-Sáinz, J.A., Hoffman, B.B., Li, S.Y., Lefkowitz, R.J. and Fain, J.N. (1980) Life Sci. 27, 953–961.
[17] Hittelman, K.J., Wu, C.F. and Butcher, R.W. (1973) Biochim. Biophys. Acta 304, 188–196.
[18] Gilman, A.G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[19] Hoffman, B.B., Mullikin-Kilpatrick, D. and Lefkowitz, R.J. (1980) J. Biol. Chem. 255, 4645–4652.
[20] DeLean, A., Munson, P.J. and Rodbard, D. (1978) Am. J. Physiol. 235, E97–E102.
[21] Hancock, A.A., DeLean, A. and Lefkowitz, R.J. (1979) Mol. Pharmacol. 16, 1–9.