INHIBITION OF CATECHOLAMINE-STIMULATED ADENYLATE CYCLASE IN FAT CELLS BY LOCAL ANAESTHETICS

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Received 23 May 1978

1. Introduction

Local anaesthetics are potent inhibitors of hormone-stimulated lipolysis in isolated fat cells [1]. This inhibition is reversible and does not concur with a reduction in intracellular 3′:5′-AMP, but rather an increase at higher concentrations of the drug [1]. Since local anaesthetics affect ion translocation [2], especially that of Ca^{2+} [3], their antilipolytic effect was interpreted as indirect evidence for a central role of Ca^{2+}-ions in the control of lipolysis [1]. This mechanism was cited as an analogy to the mechanism of action of insulin [4], where a causal connection between 3′:5′-AMP and lipolysis in the fat cell has been questioned [5,6], and where Ca^{2+} was implied as a possible messenger [4]. In view of the preliminary observation of an inhibition of catecholamine-stimulated adenylate cyclase in rat heart by lignocaine [7], it was of interest to extend this finding to the fat cell system. Because of its potent effect on lipolysis [1] tetracaine was used in most of the experiments.

2. Experimental procedure

Tetracaine hydrochloride (pantocain) was purchased from Hoechst, Frankfurt, norepinephrine bitartrate from Serva, Heidelberg, and isoproterenol from ICI-Pharma, Plankstadt. Human albumin was obtained from the Swiss Red Cross, Berne. Collagenase (lot CLSN 47B 177P) was from Worthington, creatine phosphokinase, ATP (disodium salt), cyclic 3′:5′-adenylic acid and all other biochemicals were from Boehringer Mannheim, 3′:5′-[^{3}H]AMP from the New England Nuclear Corp.; [α-^{32}P]ATP from the Radiochemical Centre, Amersham. Al_2O_3 (W 200, neutral) was from Woelm, Eschwege. All other chemicals were reagent grade from E. Merck, Darmstadt.

2.1. *Preparation of fat cells, fat-cell ghosts and fat-cell membranes*

Male Wistar rats of 120–150 g were used throughout. They had free access to normal laboratory chow and tap water. Preparation of lipocytes followed essentially the original method [8], but with a modified buffer system [9]. Incubation and determination of 3′:5′-AMP has been described [10]. Ghosts from fat cells were prepared according to [11]. Plasma membranes were partially purified from isolated fat cells on a linear sucrose gradient according to [12]. Both bands at $\rho 4^{\circ}C$ of 1.180 and 1.185 were pooled and used fresh for the adenylate cyclase assay.

2.2. *Adenylate cyclase assay*

Membrane preparations (50–100 μg protein) were incubated for 10 min at 37°C with the following additions: 20 mM Tris–HCl, pH 7.4, 4.5 mM $MgCl_2$, 1 mM 3′:5′-AMP, 10 mM creatine phosphate, 300 μg/ml creatine phosphokinase, 0.45 mM [α-^{32}P]ATP, albumin 1%, and norepinephrine–HCl and tetracaine–HCl as indicated. Termination of the reaction and separation of 3′:5′-AMP was as in [13]. Protein was determined according to [14].

3. Results

At 1 mM, tetracaine was found to inhibit the effect

Table 1
Inhibition of norepinephrine-stimulated adenylate cyclase activity in fat cell ghosts by tetracaine

Additions	Adenylate cyclase activity (nmol × g prot.$^{-1}$ × min^{-1})
None	83 ± 7
Norepinephrine, 0.1 mM	595 ± 68
Plus tetracaine, 1 mM	470 ± 50[a]

[a] $p < 0.025$, paired t-test

Values indicate mean of 4 preparations ± SEM

of 0.1 mM norepinephrine upon adenylate cyclase activity in fat cell ghosts by about 21% (table 1). Lignocaine was much less potent with an inhibition of 18% at 6.4 mM under the same conditions. In a partially-purified plasma membrane preparation a gradual inhibition of the norepinephrine-stimulated system was observed, beginning at 10^{-5} M with a

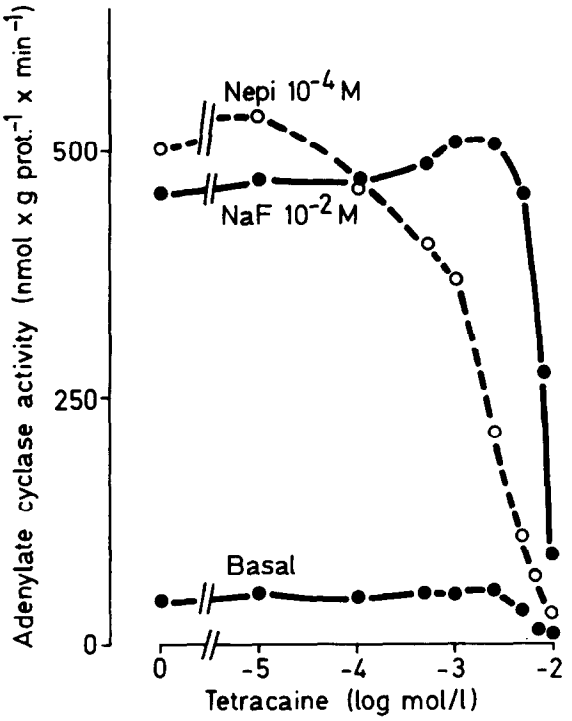

Fig.1. Inhibition of basal, norepinephrine- and NaF-stimulated adenylate cyclase activity of fat cell membranes. The reaction was started with the addition of plasma membranes.

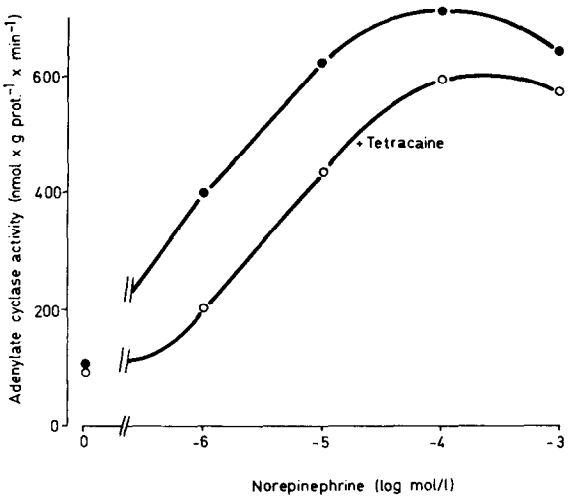

Fig.2. Dose-response curve of norepinephrine in the presence and absence of 1 mM tetracaine. Adenylate cyclase activity was measured in fat-cell ghosts.

complete inhibition at 10^{-2} M tetracaine (fig.1). In contrast, both the basal and the NaF-stimulated activity was inhibited above 5 mM. At 1 mM tetracine, a small stimulation was observed from 1–10 mM NaF (data no shown). This suggests interference with the coupling mechanism at lower, and a more direct attack at the catalytic subunit at higher, concentrations of the drug. An inhibitory effect was found throughout the dose–response range of norepinephrine (fig.2). If one compares the effect of tetracaine on lipolysis in intact cells and adenylate cyclase activity in membranes (fig.3), a considerable discrepancy is observed (app. K_i of lipolysis, 0.6 mM; app. K_i of cyclase, 2.3 mM).

In contrast to the observed inhibition of lipolysis and adenylate cyclase, no congruent drop of the levels of 3′:5′-AMP in the presence of 1 mM isoproterenol was observed (fig.4). Incubation of isolated fat cells with various concentrations of tetracaine resulted in a biphasic response of the levels of 3′:5′-AMP at 5 min. Above 0.1 mM, where inhibition of lipolysis and adenylate cyclase was found (fig.1), the cyclic nucleotide levels were above those seen with isoproterenol alone. Both, inhibition of the effect of isoproterenol at 1 μM tetracaine, and stimulation at 1 mM, were significant (fig.4).

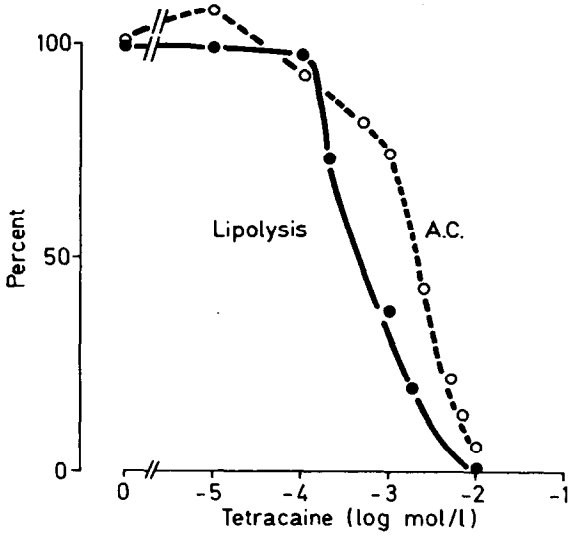

Fig.3. Comparison of tetracaine effect on norepinephrine-stimulated lipolysis in intact cells and adenylate cyclase activity in fat-cell membranes. Norepinephrine concentration in both systems: 0.1 mM; incubation time for intact cells 60 min, for cyclase assay 10 min. Maximal effect in absence of tetracaine was set at 100%.

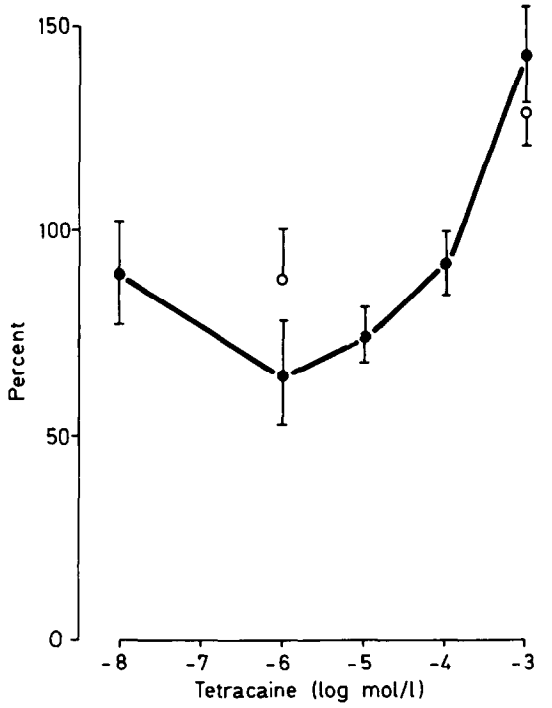

Fig.4. Effect of tetracaine on levels of 3′:5′-AMP in fat cells in the presence of 1 μM isoproterenol without (closed circles) and with (open circles) 2 mM methyl-isobutyl-xanthine. Mean of 4 experiments ± SEM the levels with isoproterenol alone (= 100%) were 27.0 ± 1.8 pmol/10^5 cells after 5 min incubation (basal levels: 3.0 ± 0.4 pmol/10^5 cells). Stimulation at 1 mM pantocaine and inhibition at 1 μM was significant in the absence of methyl-isobutyl-xanthine ($p < 0.005$, paired comparison).

4. Discussion

At 1 mM pantocaine, a significant inhibition of the norepinephrine-stimulated adenylate cyclase system is observed without any effect on the basal or fluoride-stimulated enzyme. This suggests an inhibition of signal transfer rather than a direct effect on the catalytic unit which may occur at higher concentrations (fig.1). In the light of current theories on the mechanism of action of local anaesthetics [2] the present data do not provide any clue as to the underlying mechanism. It could be due to an effect on ion translocation [15], membrane surface charge [16] or an increase in fluidity of the lipid phase from a crystalline, gel state to a liquid–crystalline state [17] with a consequent relaxation of imbedded enzyme systems. It is also known that anaesthetic amines lower the phase transition temperature of membrane phospholipids [17] which are known to play a role in transducing hormone–receptor interaction into adenylate cyclase activation [18].

It is possible that the inhibitory effect at the cyclase step is causally related to the antilipolytic action in the intact cell. The discrepancy in half-maximal concentrations does not rule out such a relationship, since broken cell preparations may exhibit lower hormone sensitivity than intact cells. In accord with a central role of 3′:5′-AMP in the control of lipolysis, one would expect a drop in this nucleotide over approximately the same concentration range of pantocaine. However, the apparent inconsistency between metabolic effects and intracellular levels of 3′:5′-AMP has already been pointed out [1], and we find the highest levels of 3′:5′-AMP (143%) at concentrations where lipolysis is strongly inhibited. Yet a lack of correlation between levels of 3′:5′-AMP and metabolic response has been observed in other situations, and compartmentation of the cyclic

nucleotide has been suggested as a possible explanation [19,20]. Thus only a small fraction of the cyclic nucleotide in the fat cell may in fact be metabolically active and responsible for the state of activation of the enzyme cascade controlling the activity of the triacylglycerol lipase.

References

[1] Siddle, K. and Hales, C. N. (1974) Biochem. J. 142, 345–351.
[2] Lee, A. G. (1976) Nature 262, 545–548.
[3] Blaustein, M. P. and Goldman, D. E. (1966) Science 153, 429–432.
[4] Kissebah, A. H., Hope-Gill, H., Vydelingum, N., Tulloch, B. R., Clarke, P. V. and Fraser, T. R. (1975) Lancet I, 144–147.
[5] Khoo, J. C., Steinberg, D., Thompson, B. and Mayer, S. E. (1973) J. Biol. Chem. 248, 3823–3830.
[6] Siddle, K. and Hales, C. N. (1974) Biochem. J. 142, 97–103.
[7] Dietze, G., Hepp, K. D. and Mehnert, H. (1972) Verh. Dtsch. Ges. Inn. Med. 78, 1113–1116.
[8] Rodbell, M. (1964) J. Biol. Chem. 239, 375–380.
[9] Heptner, W. and Christ, O. (1976) Diabetologia 3, 443–448.
[10] Hepp, K. D., Renner, R., Langley, J. and Häring, H. U. (1975) Mol. Cell. Endocrinol. 3, 309–321.
[11] Birnbaumer, L., Pohl, S. L. and Rodbell, M. (1969) J. Biol. Chem. 244, 3468–3476.
[12] Avruch, J. and Wallach, D. F. H. (1971) Biochim. Biophys. Acta 233, 334–347.
[13] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[15] Ritchie, J. M., Cohen, P. J. and Dripps, R. D. (1970) in: The Pharmacological Basis of Therapeutics (Goodman, L. S. and Gilman, A. eds) 4th edn, p. 371, Macmillan, New York.
[16] Ritchie, J. M. (1975) Brit. J. Anaesth. 47, 191–198.
[17] Papahadjopoulos, D., Jacobson, K., Poste, G. and Sheperd, G. (1975) Biochim. Biophys. Acta 394, 504–519.
[18] Birnbaumer, L. (1973) Biochim. Biophys. Acta 300, 130–158.
[19] Exton, J. H., Lewis, S. B., Ho, R. J. and Park. C. R. (1972) Adv. Cyclic Nucl. Res. 1, 91–101.
[20] Corbin, J. D., Keely, S. L., Soderling, T. R. and Park, C. R. (1975) Adv. Cyclic Nucl. Res. 5, 265–279.