HORMONE–RECEPTOR INTERACTIONS

Stimulation and inhibition of bovine adrenal cortex cell membrane adenylate cyclase by synthetic corticotropin fragments and the effect of 5′-guanylylimidodiphosphate

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1. Introduction

Recently, the differential action of corticotropin fragments on rat epididymal adipocyte membrane adenylate cyclase activity and rat adipocyte lipolysis was described [1]. It was also found in this laboratory that the benzyloxycarbonyl derivatives of two of the fragments were active in a purified preparation of bovine adrenal cortex plasma membrane vesicles containing a highly corticotropin-sensitive adenylate cyclase system and angiotensin-II-binding sites [2]. Before [3] and during these studies it became increasingly clear that high concentrations of corticotropin peptides strongly inhibit the stimulation of membrane adenylate cyclase activity.

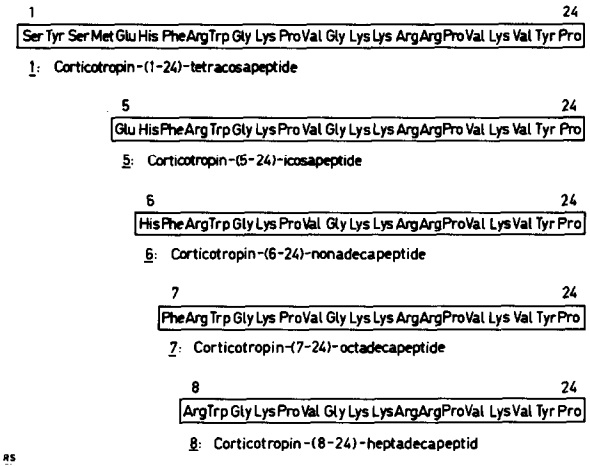
Because this type of investigation can provide new information on the mechanism of corticotropin action (review [4]), we decided to study the effects of the corticotropin peptides shown in table 1 with the bovine adrenal system. The synthesis of these peptides was described by Fauchère and Petermann [5].

Since it is known that 5′-guanylylimidodiphosphate stimulates the adenylate cyclase activity and modulates its reponses to hormones in a number of systems (review [6]), including adrenal cortex cell membranes [7], the effects of this nucleotide analogue were also investigated.

2. Experiments

The corticotropin peptides were prepared in our laboratory by classical chemical synthesis and were analytically pure [5]. The preparation of purified bovine adrenal cortex plasma membrane vesicles containing a highly corticotropin-sensitive adenylate cyclase system and angiotensin-II-binding sites were carried out according to the procedures used in our laboratory [2]. Adenylate cyclase activity was measured by a modification [2] of the method of Salomon et al. [12] with 0.5 mM ATP. We did not preincubate the vesicles with the peptides: the preparation responded immediately (see however [7]). The corticotropin peptide stock solutions were diluted shortly before application. This diminished

Table 1
Corticotropin peptides



the losses due to adsorption and hence increased the observed apparent potencies.

Experimental values were derived from 3 determinations within one and the same experiment. In fig.2, they represent the means of the values obtained from 2–3 experiments with different vesicle preparations. The 5'-guanylylimidodiphosphate concentrations varied somewhat from experiment to experiment, but were always sufficient to produce maximal effects [6].

For the purpose of comparing different experiments (as in fig.2), the adenylate cyclase activities were normalized to their basal rates, i.e. expressed as the ratio stimulated rate : basal rate.

The hormone concentration necessary for eliciting 50% of the maximal response (ED_{50}) was determined graphically.

3. Results and discussion

3.1. *Effects of 5'-guanylylimidodiphosphate (GppNHp)*

This analogue of guanosine 5'-triphosphate stimulates adenylate cyclase activity in a number of systems [6], including membrane preparations of the adrenal cortex [7]. The log dose/response curves of corticotropin peptides are sometimes modified quite considerably by the presence of GppNHp, especially with rat, less so with bovine adrenal preparations [7].

The experiments described in fig.1 reveal 3 characteristics:

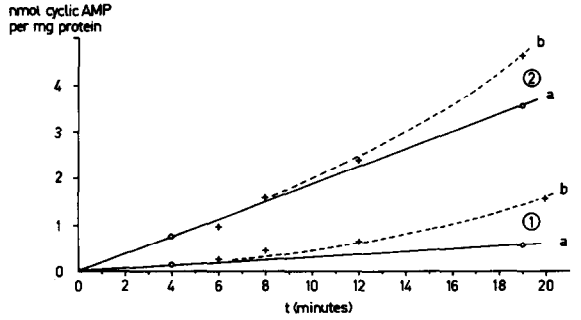


Fig.1. Influence of 5'-guanylylimidodiphosphate on bovine adrenal cortex cell membrane adenylate cyclase activity. Curves 1 without, curves 2 with 10 μM corticotropin-(1–24)-tetracosapeptide. In the experiments (b), 5'-guanylylimidodiphosphate was added at $t = 4$ min to a concentration of 36 μM, in experiments (a), the nucleotide was omitted.

(i) GppNHp enhances both the basal and the corticotropin-stimulated rates of cyclic AMP production by bovine adrenal cortex cell membrane adenylate cyclase with about equal increments.

(ii) Thus, the relative enhancement of the basal activity by GppNHp is greater than that of the corticotropin-stimulated activity.

(iii) The GppNHp effect increases with time, or, in other terms, exhibits a protracted latency period of action.

These results agree in principle with those described for other [8] and similar [7] adenylate cyclase systems.

ED_{50} was found to be $\simeq 0.05$ μM without and $\simeq 0.02$ μM with GppNHp. The slight difference is most probably insignificant. This result agrees with the observations of Glossman and Gips [7]. The modification of hormone activity produced by GppNHp in these experiments (see also fig.2) is very small compared to its modulation of β-adrenergic activity [9]. Because of the precautions taken to limit the adsorption of peptide 1 to recipients, we found ED_{50} to be about 10-times smaller than that observed previously [2] and comparable to that described elsewhere [7].

3.2. *Effects of the corticotropin analogues*

Synthetic corticotropin-(1–24)-tetracosapeptide (peptide 1) is a full agonist and at least as potent as the natural hormone, corticotropin-(1–39)-nonatriacontapeptide, in all experimental systems comprising isolated cells and cell membranes (review [4]). The shorter peptide 5, peptide 6 and peptide 7 were found to be almost equal partial agonists for rat epididymal fat cell (adipocyte) membrane adenylate cyclase activation [1,10] with potencies similar to that of peptide 1. However, despite stimulation of adenylate cyclase by all compounds, only peptide 5 stimulated lipolysis in adipocytes; the others behaved as competitive antagonists of peptide 1-stimulated lipolysis. The $N(\alpha)$-benzyloxycarbonyl derivatives of peptide 5 and peptide 7 (Z-5 and Z-7) behaved similarly. Z-5 and Z-7 were also reported to stimulate bovine adrenal cortex cell membrane adenylate cyclase activity partially [2].

Three principal features of bovine adrenal cortex cell adenylate cyclase stimulation by peptide 1, peptide 5, peptide 6 and peptide 7 immediately become apparent from the experiments summarized in fig.2:

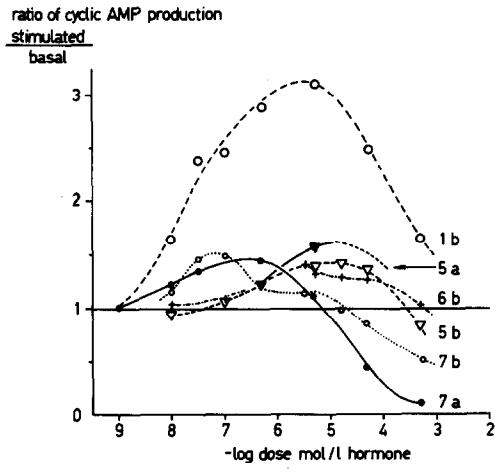


Fig.2. Log dose/response curves for the stimulation of bovine adrenal cortex cell membrane adenylate cyclase activity by synthetic corticotropin fragments numbered as shown in table 1. The results of different experiments were calculated as ratios of stimulated over basal (non-stimulated) cyclic AMP production/mg protein/15 min. Curves (a) were derived from experiments without, curves (b) from experiments with addition of 2–10 μM 5'-guanylylimidodiphosphate.

(i) The 4 peptides are active within about the same concentration range.
(ii) They all inhibit adenylate cyclase at high concentrations of the peptide, some of them even to values below the basal rate.
(iii) GppNHp has no profound influence on the behaviour of either one of these peptides.

In the presence of GppNHp, peptide 1 displays a mean maximal adenylate cyclase stimulation of 3.2-fold over the basal values. This value was taken as the reference apparent intrinsic activity ($\alpha = 1$). Its (reference) potency is in the order of 3.3×10^7 M^{-1} ($ED_{50} \simeq 0.03$ μM).

Peptide 5 is about 20-times less potent than peptide 1 ($ED_{50} \simeq 0.6$ μM) and only a 20% partial agonist ($\alpha = 0.2$) in the presence of GppNHp. In its absence, $ED_{50} \simeq 0.7$ μM and $\alpha \simeq 0.3$.

Peptide 6 is about 13-times less potent than peptide 1 ($ED_{50} \simeq 0.4$ μM) with $\alpha \simeq$ in the presence of GppNHp.

Peptide 7 is even about twice as potent as peptide 1 ($ED_{50} \simeq 0.013$ μM) with $\alpha \simeq 0.22$ both in the presence and absence of GppNHp.

Peptide 8 is inactive.

Thus, the reduced-chain peptides, with the exception of peptide 8, appear to be partial agonists for bovine adrenal cortex cell adenylate cyclase activation. Their intrinsic activities are about equal. How much importance, if any, can be attached to the observed differences between their ED_{50} values, is uncertain. More than 20-fold differences due to varying preincubation times have been demonstrated by Glossman and Struck [7]. In addition, it might well be that differential rates of adsorption to glassware, degradation rates, and inhibitory powers influence the observed ED_{50} values quite markedly. It is thus probably safe to say that the potencies of peptide 1, peptide 5, peptide 6 and peptide 7 have the same order of magnitude.

3.3. *Inhibition of adenylate cyclase activity with high concentrations of corticotropin peptides*

The inhibition of corticotropin-stimulated adipocyte membrane adenylate cyclase activity down to basal values by high concentrations of peptide 1 has been described by Lang [3]. It was shown that phenoxazones, which increase the maximal effect and the number of receptors per cell, do not influence the inhibition phenomenon. Similar effects have been observed with adrenal cortex cell membrane adenylate cyclase preparations and corticotropin peptides [2,7].

In the case of bovine adrenal cortex cell membrane vesicles (fig.2), a vesicle aggregation was often observed both visually and with dynamic light scattering (J. Morán and E. Serrallach, unpublished experiments in this laboratory). The questions arised as to whether the inhibition was reversible and to whether the aggregation is responsible for the inhibitory effect.

Figure 3 shows that a 25-fold dilution from 250–10 μM peptide 1 almost fully restores the activity. The aggregation of the vesicles, however, remained visibly unchanged. Addition of GppNHp without dilution of the hormone was ineffective.

4. Conclusions

The membrane adenylate cyclase activities of bovine adrenal cortex and of rat epididymal fat pads respond very similarly towards the peptides examined here. The adrenal system is strongly stimulated by corticotropin-

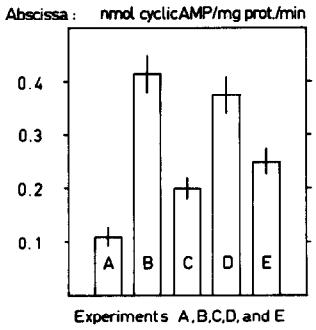


Fig.3. Inhibition and restoration of bovine adrenal cortex cell membrane adenylate cyclase activity. (A) Non-stimulated, basal activity. (B) Stimulation by 10 μM peptide 1. (C) Stimulation by 250 μM peptide 1 after preincubation for 5 min with 500 μM hormone. (D) Stimulation by 10 μM peptide 1 after preincubation as in (C). (E) As in (C) but with addition of 50 μM 5'-guanylylimidodiphosphate after the preincubation with hormone.

(1–24)-tetracosapeptide (peptide 1) and partially so by peptides with shorter N-terminal sequences: corticotropin-(5–24)-icosapeptide (peptide 5), corticotropin-(6–24)-nonadecapeptide (peptide 6), and corticotropin-(7–24)-octadecapeptide (peptide 7). However, the peptide lacking phenylalanine, corticotropin-(8–24)-heptadecapeptide (peptide 8) is inactive. The responses of the adrenal enzyme are only slightly modulated by 5'-guanylylimidodiphosphate.

The strong inhibition of stimulated (and even basal) membrane adenylate cyclase activity by high corticotropin peptide concentrations (10–250 μM peptide 1, peptide 5, peptide 6 and peptide 7) is a property common to both systems. Its mechanism remains unknown. As a consequence, great care must be exercised when interpreting data from inhibition experiments: an agonist at low concentrations ($< 1-10$ μM) will inevitably turn into an antagonist at high concentrations (> 10 μM) and any slight intrinsic stimulatory activity might become almost completely obscured by the inhibitory effect.

These apparently quite general inhibitory properties may help to explain the recent observations of Finn et al. [11] that certain corticotropin analogues which, at concentrations of 10–180 μM, antagonize the action of peptide 1 upon bovine adrenal cortex cell membrane adenylate cyclase are nervertheless able to elicit cyclic AMP accumulation and steroidogenesis in whole cells.

The implications of this experimental work for the theories of one-dimensional organization of information in corticotropin [4] and of mediation by cyclic AMP shall be dealt with elsewhere [10].

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References

[1] Lang, U., Fauchère, J. L., Pelican, G. M., Karlaganis, G. and Schwyzer, R. (1976) FEBS Lett. 66, 246–249.
[2] Schlegel, W. and Schwyzer, R. (1977) Eur. J. Biochem. 72, 415–424.
[3] Lang, U. (1973) Dissertation No. 5215, ETH Zürich; Lang, U. and Schwyzer, R. (1976) in: Peptide Hormones (Parsons, J. A. ed) pp. 337–348, Macmillan Press Ltd, London.
[4] Schwyzer, R. (1977) Annals NY Acad. Sci. in press.
[5] Fauchère, J.-L. and Petermann, C. (1977) Helv. Chim. Acta in press.
[6] Rodbell, M., Lin, M. C., Salomon, Y., Londos, C., Harwood, J. P., Martin, B. R., Rendell, M. and Bermon, M. (1975) Adv. Cyclic Nucleotide Res. 5, 3–29.
[7] Glossmann, H. and Gips, H. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 286, 239–249; (1975) 289, 77–97; Glossmann, H. (1975) ibid. 289, 99–109; (1975) 291, 89–100; Glossmann, H. and Struck, C. J. (1976) ibid. 294, 199–206.
[8] Rodbell, M. (1975) J. Biol. Chem. 250, 5826–5834; Salomon, Y., Lin, M. C., Londos, C., Rendell, M. and Rodbell, M. (1975) ibid. 4239–4245.
[9] Lefkowitz, R. J., Mullikin, D. and Caron, M. G. (1976) J. Biol. Chem. 251, 4686–4692.
[10] Schwyzer, R. et al., in preparation.
[11] Finn, F. M., Johns, P. A., Nishi, N. and Hofmann, K. (1976) J. Biol. Chem. 251, 3576–3585.
[12] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.