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INFLUENCE OF ENKEPHALINS ON THE ACTH-INDUCED HORMONAL IMPRINTING IN TETRAHYMENA

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Both adrenocorticotrop hormone (ACTH) and the synthetic enkephalins investigated evoked imprinting in Tetrahymena and led to increased hormone binding at further contact with ACTH. Neither molecule evoked, however, imprinting for the enkephalins. The pentapeptide enkephalin containing also proline had the most pronounced imprinting effect and, when given together with ACTH, it increased the imprintatory effect of ACTH considerably. In all the situations investigated the enkephalin tetrapeptide inhibited the positive effect of the enkephalin pentapeptide, whereas it did not influence the imprintatory effect of ACTH. Similarities can be found between the pharmacological and imprinting effects of enkephalin in mammals, and the effects seen in the present investigations.

Keywords: enkephalins, ACTH, hormonal imprinting, Tetrahymena, receptor development

During hormonal imprinting a certain hormone influences the cell and this influence leads to the development of a special memory [4, 5]. As a consequence of the development of this special memory, the cells, or their descendant cells, at further contact with the hormone will exhibit altered, usually enhanced response capability [7]. Neither the exact cause of the phenomenon, nor the mechanism of the fixation of the memory is known. Nevertheless, it has been demonstrated that, the intactness of the outer surface membrane of the cell and certain intracellular mechanisms as well as the intact functional state of the nucleus are both prerequisites of the development of imprinting [13, 14].

In the case of peptide hormones both the actual condition of the cellular membrane and the qualitative and quantitative characteristics of the binding sites of the membrane are of decisive importance. The specificity of hormone receptors is based on the close interrelated and interdetermined connection between the hormone and the binding site [3, 16]. This connection has often been characterized by the key-lock symbol. The experiments which investigate

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the effect of substances of quasi-similar structure render easier the exact characterization of the receptors also from this point of view. In these experiments such alterations can be found both in the response capability of the cell and in hormonal imprinting itself which may be explained by the selectivity of the membrane at the molecular level [10]. Such experiments make it possible to characterize a certain molecule also from point of view, which part of the molecule participates in the receptor-hormone interaction with the receptor, since the homologous receptors which develop as a result of imprinting are able to bind only substances which have active sequences. Under such conditions those possibly related molecules can also be indentified which, on the one hand, are able to evoke the above-characterized process of receptor amplification in spite of their more or less different structure, or, on the other hand, which do not meet the biochemical-stereochemical requirements to evoke imprinting, in spite of their similarity to the active structure.

In the present experiments adrenocorticotrop hormone (ACTH) and two enkephalin derivates were investigated, i.e. substances containing identical and different amino acid sequences. Tetrahymenas were used as model cells for studying the influence of the above-mentioned substances on the extent of the changes of the response capability of the cells and on the possible development of cross-reactions between the substances investigated.

Materials and methods

Tetrahymena pyriformis GL strain in the logarithmic phase of proliferation was maintained at 28 °C in Bacto trypton medium (Difco, Michigan, USA) containing also 0.1% yeast extract. The cells were incubated with the following substances (concentrations are shown in parentheses) for 60 minutes: ACTH (Exacthin, Richter, Budapest, 10^{-6}M), Des-Tyr–l, (D-Met 2-, Pro 5)-Enkephalin-amide (E–l, 10^{-6}M) and Tyr-D-Met-Gly-Phe-NH_2-AcOH (E2, 10^{-6}M), both enkephalins were synthesized by S. Bajusz, Institute for Drug Research, Budapest.

The cells were washed in phosphate buffer (PBS, pH 7.2) and treated in groups as follows: control, ACTH, ACTH+E1, ACTH+E2,El+E2, El and E2.

Twenty-four hours after the treatment the cells were inoculated to normal medium. After 9 or 10 descendant generations the cells were fixed in 0.4% formol solution dissolved in PBS and incubated for 60 minutes with the fluoresceine isothiocyanate (FITC, BDH, London, England)-labelled form of the substances used for the first incubation. Then the cells were washed in PBS three times and dropped to slides.

The extent of the binding of the FITC-labelled substances was measured by a Zeiss Fluoval cytofluorimeter. Data were analyzed by means of HP-41CX micro-computer attached to the fluorimeter. The fluorescence of 20–20 cells was measured in each group and the experiments were repeated three times independently, thus, a column on the Figure represents the mean value of 60 cells. The significance level of the results obtained was evaluated by Student's *t* test.

Results and discussion

Adrenocorticotrop hormone, beta-lipoprotein and enkephalins are related hormones which are secreted at the same site and which contain, in certain regions, identical amino acid sequences [3, 14]. Therefore also some functionally

overlapping effects can be detected. In the present experiments ACTH and the two synthetic enkephalin derivates were investigated in order to identify those structures which are able either to develop receptors for each other or to inhibit (disturb) receptor formation. Since in Tetrahymena the membrane structures which are nonspecifically present strenghten and develop into receptors but in the presence of the hormone [6], there the imprinting evoked in Tetrahymena seems to be a suitable model system for the investigation of the problem.

The results obtained unequivocally indicate that all the three substances investigated strenghtened (imprinted) receptors in the Tetrahymena for ACTH (Fig. 1), meanwhile neither of the substances was able to develop receptors for the enkephalin derivates (Figs 2 and 3). As far as imprinting for ACTH was concerned, it was ACTH itself which had the weakest, though significant effect. The effect of the first enkephalin (E2 = Tyr-D-Met-Gly-Phe-NH_2) was more pronounced, while the most pronounced effect was seen in the case of the other enkephalin (E1-Tyr-D-NLe-Gly-Phe-Pro-NH_2). This finding is quite surprising if either the difference between the size of the molecules or the relative diversity of the amino acid sequences is considered. Moreover, it is presumably the Met-Glu-His-Phe-Arg-Trp-Gly heptapeptide which is responsible for the activation of the ACTH receptor (at least in mammalian suprarenal gland) [12], and this heptapeptide is not present in this form in the enkephalins. Nevertheless, we should accept the finding since the same result was seen in the repeated experiments, too.

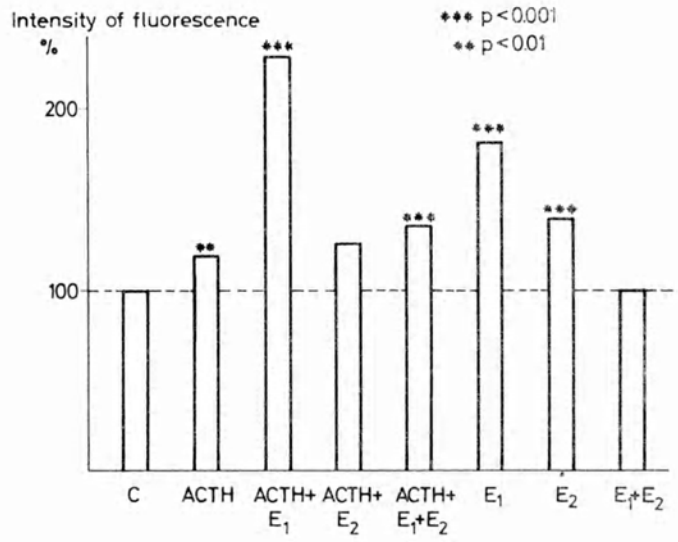

Fig. 1. Effect of single and combined imprinting by ACTH and enkephalins (El and E2) on the ACTH binding

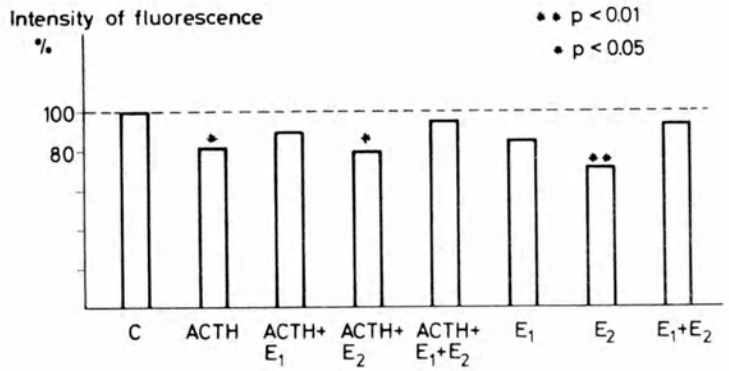

Fig. 2. Effect of single and combined imprinting by ACTH and enkephalin (E1 and E2) on the E1 enkephalin binding

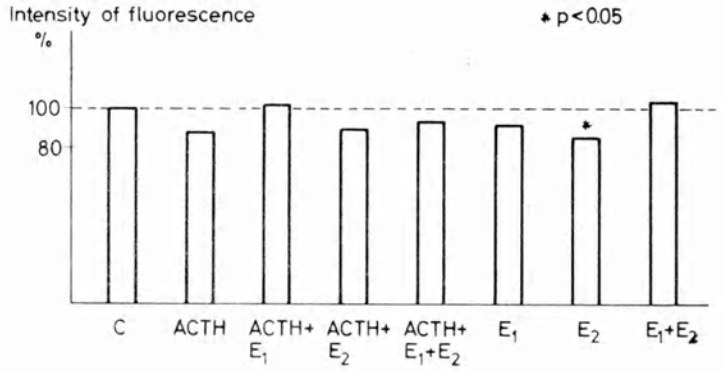

Fig. 3. Effect of single and combined imprinting by ACTH and enkephalins (E1 and E2) on the E2 enkephalin binding

When imprinting was evoked by the simultaneous administration of more than one molecule it became clear that while the E2 enkephalin practically did not influence the imprinting effect of ACTH, the E1 enkephalin increased considerably the imprinting effect (to such extent which exceeded any other imprinting effect). On the other hand, the simultaneous presence of E1 and E2 molecules had negative influence in all the situations investigated, decreased the pronounced effect of the ACTH + E1 combination and decreased the imprintatory effect of the E1 molecule practically to the control level.

The pronounced imprintatory effect of the E1 molecule can be explained. The *in vivo* effectivity of the E1 molecule compared to enkephalin is 64.6, whereas that of the E2 molecule is only 12.1. [1, 2]. When it was investigated in the Chang liver cell culture model the imprintatory effect of the E1 molecule was 248% (control = 100%) while the E2 molecule evoked negative impriting

and reached only 78.6% of the control value [10]. Moreover, the E1 molecule contains proline which has higher receptor affinity than that of the other amino acids [11, 12]. These considerations would explain the fact that the E1 molecule evoked more pronounced impriting for ACTH than the other molecule did and increased the influence of ACTH, if it could have simultaneously evoked marked imprinting for itself, too. This latter effect, however, was seen neither in previous investigations, nor in the present study. Therefore a positive receptor cooperativity may also be assumed which would enhance ACTH binding and which could be inhibited by the negative cooperativity of the E2 molecule. All these considerations, however, are only theoretical assumptions which clearly need corroboration. Nevertheless, it is a striking finding that the negative effect (negative cooperativity) of the E2 molecule manifested itself only in the presence of the E1 molecule, but not in the presence of ACTH.

The more pronounced effect of the alien (related) imprinting than that of the own imprinting had been demonstrated also in previous investigations carried out on mammalian cells [8, 9]. The phenomenon could also be demonstrated, e.g. in the gonadotropin-thyrotropin relation, either cell lines or perinatal imprinting was investigated. The present observation, however, seems to be novel from that point of view that the molecules investigated (enkephalins) evoked imprinting only for the related molecule but not for themselves, whereas they markedly influenced the imprinting effect of both the other enkephalin and ACTH. Moreover, considering that the relationship between ACTH and the synthetic enkephalins investigated in the present study is rather indirect [14], the phenomenon observed seems to be even more strange.

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