Acta Medica Okayama

Volume 39, *Issue* 4 1985 *Article* 7

AUGUST 1985

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Age-associated changes in methionine-enkephalin (ENK) and thyrotropin releasing hormone (TRH) concentrations, and their receptors were examined in discrete regions of the rat brain. The ENK and TRH concentrations in aged rats were nearly identical to those in young adult rats, except for a slightly lower TRH value in the hypothalamus of the aged rats. On the other hand, the ENK and TRH receptor levels in the cerebral cortex of aged rats was markedly lower than that of young adults rats. The results suggest that determinations of both neuropeptide and receptor levels are indispensable for evaluation of peptide-mediated neural systems in the central nervous system.

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*PMID: 2996307 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **39,** (4), 315-319 (1985)

NEUROPEPTIDES AND THEIR RECEPTORS IN AGED-RAT BRAIN

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Received February 18, 1985

Abstract. Age-associated changes in methionine-enkephalin (ENK) and thyrotropin releasing hormone (TRH) concentrations, and their receptors were examined in discrete regions of the rat brain. The ENK and TRH concentrations in aged rats were nearly identical to those in young adult rats, except for a slightly lower TRH value in the hypothalamus of the aged rats. On the other hand, the ENK and TRH receptor levels in the cerebral cortex of aged rats was markedly lower than that of young adult rats. The results suggest that determinations of both neuropeptide and receptor levels are indispensable for evaluation of peptide-mediated neural systems in the central nervous system.

Key words : methionine-enkephalin (ENK), thyrotropin releasing hormone (TRH), receptors, aged-rat brain.

In the central nervous system (CNS), transmission of information is mediated by chemical substances called neurotransmitters. Neurotransmitters, which may be amino acids, amines or neuropeptides, have been studied together with their receptors. Although the knowledge about amines and amino acids has greatly increased, information on neuropeptides is still limited despite the rapid advances in research. Monoamines are 1,000 times more concentrated than neuropeptides in the CNS (1). The finding that neuropeptides have biological activities at such low concentrations suggests that these peptides may play an important role in the CNS.

Studies of neuropeptides mostly have been limited to measurements of their concentrations by radioimmunoassay (RIA). However, these concentrations are dependent on the synthesis, storage and release of the peptides. Increases in the values do not necessarily mean enhanced function, since cessation of release could cause such increases. The function of neurons subserved by amines may be evaluated by simultaneously measuring the concentration of amines and their metablites. The neural function mediated by peptides, on the other hand, cannot be accurately estimated by RIA because peptide fragments are unavoidably included in the measurements. Therefore, for accurate assessment of the function of neurons in the peptide system, the receptor levels must be measured along with neuropeptide levels. If peptide release increases, the receptors exclusively present

in the synapse will be down-regulated with a resultant decrease in receptor binding on exposure to the peptide. If the peptide release ceases, the receptors would be expected to be up-regulated and receptor binding to be increased (2). In the present study, therefore, age-associated changes in both neuropeptide concentrations and their receptors were examined in the rat. Methionine-enkephalin (ENK) and thyrotropin releasing hormone (TRH) were studied because they are considered to be closely associated with emotional activity and consciousness.

MATERIALS AND METHODS

ENK and TRH were purchased from Protein Research Foundation, Osaka, Japan. ^{125}I-ENK and [^{3}H]ENK were purchased from Amersham, Buckinghamshire, U.K., and ^{125}I-TRH and [^{3}H]TRH were from New England Nuclear, Boston, MA, U.S.A. Male Fisher rats were obtained from Charles River Japan Inc.

Aged (24-month-old) and young adult (8-week-old) male rats were decapitated, and the brains were divided according to the method of Glowinski and Iversen (3). For the RIA, brain tissue was homogenized in 10 volumes of acidified ethanol (0.1 N HCl : ethanol = 1 : 1); the homogenate was centrifuged at 12000 xg for 20 min according to a previously reported method (2), and the resulting supernatants were dried at 40 ℃ under continuous N_2 gas stream and then stocked at -20 ℃ until RIA. The recovery of ENK and TRH added in the extraction medium before the homogenization was 80 % and 87 %, respectively.

Radioimmunoassay (RIA). The diluent for the reagents and samples was 0.14 M sodium phosphate buffer, containing 25 mM EDTA and 0.5 % BSA, pH 7.4.

ENK-RIA. Antibodies to synthetic ENK were produced in rabbits affter conjugation of ENK to bovine serum albumin (BSA), similarly to the method for producing anti-β-endorphin antibodies (4). The anti-ENK-serum used in the present study bound 40 % of the tracer at a final dilution of 1 : 3,000. The antiserum crossreacted lower than 0.1 % with leucine-enkephalin, and no cross reactivity was evidenced with α-, β- and γ-endorphin, ACTH, somatostatin, TRH and LH-RH. To each assay tube was added 0.2 ml of the diluent, 0.1 ml of anti-ENK rabbit serum (final concentration 1 : 3,000), 0.1 ml of ENK solution (in concentrations from 0.5 ng/ml to 1,000 ng/ml) or samples, and 0.1 ml of ^{125}I-ENK (15,000 cpm). This reaction mixture was incubated at 4 ℃ for 48 hr, then 0.1 ml of 0.2 % human γ-globulin and 0.6 ml of 25 % polyethylene glycol were added. Each assay tube was thoroughly vortexed and left for 20 min in ice. The tubes were then centrifuged at 3,000 rpm for 30 min and then decanted. The pellet was counted for radioactivity with an automatic gamma counter. The intra- and inter-assay coefficients of variation of ENK-RIA were 4.2 % and 11.0 %, respectively.

TRH-RIA. The TRH-RIA was conducted by a previously described method (2).

Radiolabeled Receptor Assay (RRA). The samples were homogenized in Tris-HCl buffer, and 50,000 xg pellets were prepared as described previously (5). Samples of each receptor preparation (500 μg protein) were incubated in ice for 120-180 min with 4 nM [^{3}H]ENK or 12 nM [^{3}H]TRH in the presence or absence of unlabeled ENK (10 μM) or TRH (100 μM). Specific binding was the difference between the radioactivity bound to the receptor in the presence of 10 μM ENK or 100 μM TRH and in their absence (5, 6).

Saturation experiments were carried out using the cerebral cortex. Cerebral cortex homogenate suspension (containing 700 μg protein in Tris-HCl buffer) was incubated in ice for 120-180 min with increasing concentrations of [^{3}H]ENK (2-35 nM) or [^{3}H]TRH (1-40 nM)

in the presence or absence of unlabeled 10 μM ENK or 100 μM TRH in a total volume of 1 ml.

RESULTS AND DISCUSSION

The upper penel of Fig. 1 summarizes the ENK and TRH concentrations in

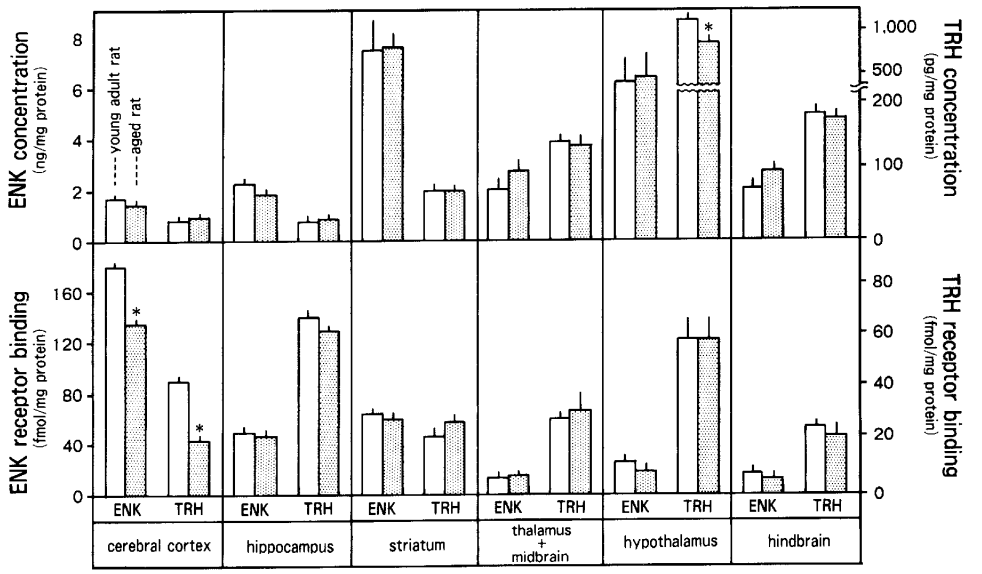

Fig. 1. ENK- and TRH-concentration (upper panel) and their receptor bindings (lower panel) of the aged-rat brain. The data are shown as the mean + SEM of 5 rats. ☐, young adult rats ; ▨, aged rats. *, $p<0.05$ compared to young adult group.

different areas of the brain as determined by RIA. The ENK and TRH concentrations in aged rats were nearly identical to those in young adult rats, except for a slightly lower TRH value in the hypothalamus of the aged rats. The results suggest that aging apparently has no effect on the ENK- or TRH-mediated neuronal system. Age-associated changes in ENK and TRH receptor levels were examined in different areas of the brain, and the results are shown in the lower panel of Fig. 1. The ENK receptor level in the cerebral cortex of aged rats was markedly lower than that of young adult rats, and the TRH receptor level in the same region was half that of young rats. These results suggest that at least a part of the supposed dysfunction of psychomotor activity such as learning, memory and consciousness in aged rats is due to the decrease in ENK and TRH receptor binding in the cerebral cortex. ENK and TRH receptor binding levels in aged rats were identical to those in young adult rats in 5 other regions examined.

Fig. 2 shows the Scatchard plots of saturation experiments using the cerebral cortex. As shown in the left panel of Fig. 2, the decrease in ENK binding in

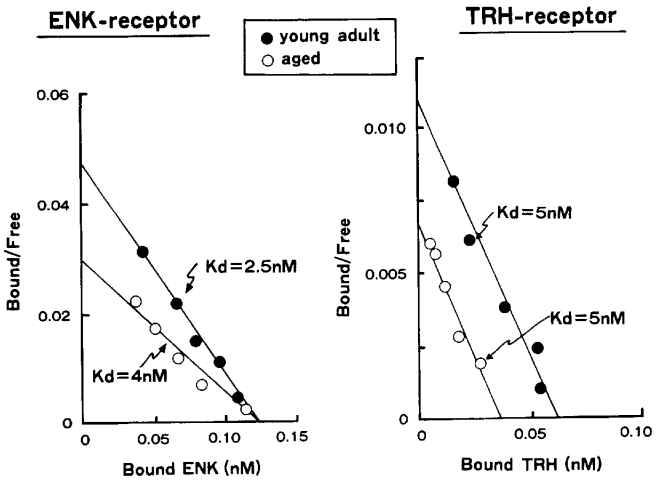

Fig. 2. Scatchard plot of ENK receptor binding (left panel) and TRH receptor binding (right panel) of young adult and aged-rats. Saturation of [^{3}H]ENK binding and [^{3}H]TRH binding to homogenates of the cerebral cortex were performed as described in Materials and Methods.

cerebral cortex is due to the decreased affinity of the binding sites. The reduced TRH receptor activity in the cerebral cortex of aged rats resulted from the decrease in the number of receptors, and not from changes in their affinity (right panel of Fig. 2).

As demonstrated in the above experiments, determination of neuropeptide levels is not sufficient for the study of age-associated changes in the peptide-mediated neural system of the CNS. The effects of some agents on peptide-mediated neural systems can become clear only after measurements of the receptor levels (7). Although determinations of both peptide and receptor levels are indispensable for the evaluation of synaptic activities, the latter levels are often more sensitive.

Acknowledgment. This work was supported in part by a research grant from the Japanese Ministry of Education, Science and Culture (No. 59125001).

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