COMPARISON OF NEURO- AND IMMUNOMODULATOR PROPERTIES OF LOW-MOLECULAR-WEIGHT NEUROPEPTIDES

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KEY WORDS: low-molecular-weight neuropeptides; neuromediators; immunomodulation; humoral antibodies.

Much evidence has now been obtained on the regulatory role of natural low-molecular-weight peptides. There are conclusive data on their effect on behavioral, nociceptive, and glucoregulatory responses [3], on the brain level of biogenic monoamines [2], and on the synthesis and secretion of various hormones [8]. Considering these multiple functions of the peptides, it was interesting to obtain information on the effect of neuropeptides on immune reactions *in vivo*. This paper gives the results of an investigation and comparison of the neuro- and immunomodulating action of low-molecular-weight neuropeptides.

EXPERIMENTAL METHOD

The effect of the neuropeptides Leu- and Met-enkephalins, thyroid hormone releasing factor (THRF), the C-terminal tripeptides of gastrin (MAF) and oxytocin (MIF) on the brain level of biogenic monoamines and their metabolites and on production of humoral antibodies against sheep's red blood cells (SRBC) was studied. The action of the above-mentioned peptides was compared with the action of an immunostimulator of peptide nature, namely, tuftsin.

BALB/c mice and Wistar rats were used. The concentrations of noradrenalin (NA), dopamine (DA) and its metabolite homovanillic acid (HVA), and serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined in the brain of the decapitated mice and rats spectrofluorometrically [9] 5 min after intracisternal injection of THRF (200 μg per animal) and intraventricular injection of the peptides. For intraventricular injections, doses were chosen (Table 1) which produced the most marked behavioral responses [7]. The concentration of biogenic monoamines was calculated in per cent of the control (animals receiving physiological saline).

SRBC were injected intraperitoneally into mice in a dose of 5×10^7 cells in 1 ml physiological saline 3 days after injection of the peptides. All peptides except THRF were dissolved in physiological saline and injected intravenously as a single dose of between 5 and 100 mg/kg body weight. When the dose range was chosen, consideration was paid to data in the literature according to which doses of 10-20 mg/kg are needed for immunostimulation *in vivo* by tuftsin. THRF was tested in doses of 10 μg/kg to 150 mg/kg for intravenous injection and from 20 to 400 μg per animal for intracisternal injections.

In experiments to study interaction of THRF with haloperidol and MAF, the haloperidol was injected intraperitoneally into the mice in a dose of 5 mg/kg, 15 min before intravenous injection of THRF, whereas MAF was injected intravenously in a dose of 10 mg/kg simultaneously with THRF. In both cases, THRF was injected intravenously in a dose of 100 mg/kg. Control animals were given an injection of physiological saline at the same times and in the same volumes as the test substance.

Blood was collected from the orbital venous sinus on the 7th day after immunization. The titer of hemagglutinating antibodies was determined in plates of a Takachi microtitrator. The experimental data was subjected to statistical analysis at a 95% level of significance.

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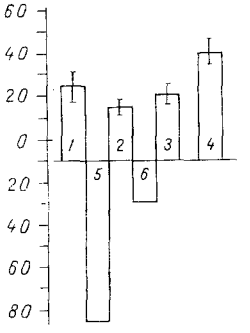
TABLE 1. Effect of Peptides on Content of Brain Biogenic Monoamines (%) following Intraventricular Injection into Rats (M ± m, n = 12)

Substance	Dose, μg per animal	NA	DA	HVA	5-HT	5-HIAA
Physiological saline	—	100±9	100±7	100±10	100±14	100±13
MAF	200	105±10	59±6*	69±12*	157±17*	135±15*
THRF	20	124±10*	144±12*	146±12*	143±12*	135±13*
THRF (intracisternal injection into mice)	200	103±8	133±13*	154±11*	140±7*	188±6*
THRF + MAF	20+200	93±13	92±8	90±20	153±14*	129±7*
Leu-enkephalin	200	84±12	95±16	109±12	95±15	128±5*
Met-enkephalin	200	100±19	94±13	88±7	102±13	143±10*
MIF	100	110±13	143±13*	135±8*	90±7	95±12
Tuftsin	100	90±4	91±16	96±15	118±18	104±8

*P ⩽ 0.05 relative to control.



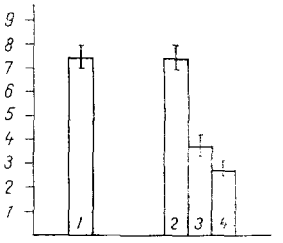


Fig. 1 Fig. 2

Fig. 1. Effect of peptides on titer of hemagglutinating antibodies in mice. Ordinate: above zero level — increase, below — decrease (in % relative to control). 1) Tuftsin (20 mg/kg), 2) Leu-enkephalin (20 mg/kg), 3) MAF (10 mg/kg), 4) MIF (20 mg/kg), 5) THRF (100 mg/kg), 6) Leu-enkephalin (100 mg/kg). Differences in all cases statistically significant (P ⩽ 0.05).

Fig. 2. Effect of THRF on immune response when given by intracisternal injection. Ordinate, antibody titer ($\log_2$). 1) Control, 2) THRF in dose of 100 μg per animal, 3) 200 μg, 4) 400 μg.

All peptides except enkephalins were synthesized at the Experimental Factory of the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR; Met-enkephalin was obtained from Fluka, Switzerland.*

EXPERIMENTAL RESULTS

The neuropeptides chosen for study, which were previously shown to have central effects [1, 3, 7], modified the synthesis or turnover of brain biogenic monoamines (Table 1), and affected chiefly dopaminergic and serotoninergic systems. Tuftsin, whose action in the above-mentioned doses when given by intraventricular injection, as our results showed, was not reflected in the total content of biogenic monoamines, nevertheless interferes with catecholaminergic processes, as was shown previously [1], when its administration in this way caused changes in brain tyrosine hydroxylase activity.

All the peptides studied except Met-enkephalin had an immunomodulating action. MAF and MIF in doses of 10 and 20 mg/kg had a stimulating effect (Fig. 1), but these doses are a little higher than those (5 mg/kg) which caused neurochemical changes on parenteral injection [2]. In other doses, these peptides did not significantly alter the level of the immune response in

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mice. Leu-enkephalin in doses of 10 and 20 mg/kg raised the titer of humoral antibodies, but in a dose of 100 mg/kg it had a slight but statistically significant inhibitory action on synthesis of antibodies against SRBC (Fig. 1).

THRF had the most individually distinct action. In doses of 10 μg to 50 mg/kg, in which marked cytotropic effects were manifested, the content of biogenic monoamines in the brain was affected but not the immune response. Only in doses of 100 and 150 mg/kg did it reduce the antibody titer. This peptide inhibited antibody production not only after intravenous, but also after intracisternal injection, when access of the peptide to the immunocompetent cells of the blood and lymphoid tissue was difficult, but contact with the neurotransmitters of the brain was facilitated. Starting with a dose of 200 μg per animal, THRF considerably inhibited antibody formation (Fig. 2). The content of DA and its metabolite EVA, and also 5-HT and its betabolite 5-HIAA was increased (Table 1). The results suggest that the action of THRF and of other peptides on the intensity of antibody synthesis is mediated through the neurotransmitters of the brain. This conclusion is confirmed by data showing that injection of neurotransmitters into animals affects antibody formation in them, which it either stimulates or inhibits [6]. It has also been shown that membranes of immunocompetent lymphocytes and macrophages, which have a set of receptors of varied specificity, also have receptors for cholinergic and adrenergic substances [10].

THRF has a stimulating action on the CNS, due at least in part to activation of the dopaminergic system of the brain [5, 7]. Considering this fact, it can be postulated that inhibition of antibody synthesis by large doses of THRF may be due to its action on dopamine receptors, which are blocked by haloperidol. Preliminary administration of haloperidol in fact abolished the immunosuppressive action of THRF. The antibody titer in the control animals and in animals receiving THRF and haloperidol was 6.7 ± 0.25 and 7.4 ± 0.39, respectively, whereas the mean geometric antibody titer in mice receiving THRF only, in a dose of 100 mg/kg, was 5.0 ± 0.35.

Meanwhile, MAF, which prevents the psychostimulant action of THRF [4] and reduces the rise in the level of DA and HVA induced by THRF (Table 1), did not abolish the fall in the hemagglutinin level under the influence of THRF. The antibody titer in the animals of this group remained almost the same as in mice receiving THRF only (4.7 ± 0.3). In other words, despite the fact that MAF in its sedative properties resembled haloperidol to some extent, and that psychotropic interaction between THRF and MAF took place at the level of dopamine receptors [2, 4], no antagonism was exhibited in their immune reactions.

Low-molecular-weight peptides which have a neuromodulating action can thus also affect the character and intensity of the immune response. This effect is mediated, perhaps in part, by neurotransmitters, for which specific receptors are found on the cell membrane of immunocompetent cells. However, no direct correlation exists between the immunopharmacologic characteristics and neurochemical parameters of activation of brain monoaminergic systems by neuropeptides. Each peptide, depending on its structural characteristics, evidently produces different changes in the pathway of synthesis and metabolism of brain biogenic monoamines and has different effects on cellular processes involved in regulation of the immune response of the body.

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EFFECT OF HALOPERIDOL ON DISTRIBUTION OF FUNCTIONALLY DIFFERENT CELLS IN IMMUNOCOMPETENT ORGANS

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Activation of the serotoninergic system by administration of the amine itself and of substances affecting its metabolism, has been shown to cause suppression of the immune response of IgM and IgG [9]. The same effect may also be achieved by administration of haloperidol (HP) [14], a specific blocker of dopamine receptors, whereas activation of the dopaminergic system [2] leads to stimulation of the immune process.

The action of serotonin on immunogenesis has been found to be due to a redistribution of cell populations possessing suppressor activity in both central and peripheral immunocompetent organs [4].

The object of this investigation was to study the principles governing the distribution of lymphocytes when the immune response is depressed by HP, i.e., during inhibition connected with another (dopaminergic) amine system, in order to determine the basis of suppression of immune responses during blockage of that system.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice aged 2 months in a system of syngeneic cell transfer: cell suspensions from intact (control) and HP-treated animals were transplanted into lethally irradiated recipients (800 R) together with sheep's red blood cells (5×10^6). Spleen cells were transplanted in a concentration of 40 million cells per mouse, cells of other organs in a concentration of 10 million per mouse. The donors received a single injection of HP in doses of 5 and 12 mg/kg subcutaneously in physiological saline 2 h before removal of the organs.

The intensity of the immune response in the recipients was determined in the spleen by the number of rosette-forming cells (RFC) on the 5th day after immunization [5]. IgM- and IgG-RFC were differentiated by their sensitivity to 2-mercaptoethanol [13].

EXPERIMENTAL RESULTS

Transplantation of spleen cells alone and also together with lymph node cells from donors receiving HP in a dose of 5 mg/kg stimulated the recipients' immune response compared with the control. Whereas in the first case stimulation was due to an increase in the level of both IgM-RFC and IgG-RFC, addition of lymph node cells caused the increase to be chiefly confined to the IgG-RFC (Fig. 1A). Meanwhile, combined transplantation of spleen and bone marrow cells from animals receiving HP revealed a marked decrease in rosette formation in the recipients on account of both types of RFC compared with this parameter when a similar cell suspension obtained from intact donors was injected. Simultaneous transplantation of spleen and thymus cells from donors receiving HP did not change the total RFC level in the recipients compared with the control, but did cause it to decrease compared with transplantation of spleen cells alone from these donors. In this situation, there was a sharp decrease in the number of IgM-RFC and an increase in the number of IgG-RFC.

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