PORCINE BRAIN CORTEX NEUROPEPTIDES ENHANCE THE EARLY PHASE OF ANTIBODY SYNTHESIS IN THE VACCINATED MOUSE

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KOKKUVÕTE: Sea peaajukoore neuropeptiidid suurendavad varajases sünteesi faasis antikehade moodustumist vaktsineeritud hiirtel. *Töös uuriti sea peaaju neuropeptiidide mõju immuunsüsteemile valge hiire populatsioonis. Tuvastati neuropeptiidide, eriti Pld, manustamisel antikehade tiitri suurenemine vereseerumis antikehade varajases sünteesi faasis.*

Abstract

Applied biologists are confronted to the tendency to relieve antibiotics, which is growing every year. Therefore the research of new drugs inclusive neuropeptides and the potentiation of vaccines are needed. Likewise, there does not exist any survey on porcine brain chemoarchitecture. As the experimental animal, the recipient of neuropeptides was the genetically heterogeneous Swiss white mice population. The brain cortex donors were adult Estonian bacon breed pigs. In the paper the following study model was used: porcine cerebral cortex neuropeptides – vaccination with *Salmonella typhimurium* strain 34–96 vaccine. The subcutaneous dose of neuropeptides was 66–70 ng. It was demonstrated that both studied porcine neuropeptides exhibited an immunostimulant activity. The vaccine and neuropeptides were challenged simultaneously. The preparations exhibited a stimulating action to early antibody synthesis at 5^{th}, 7^{th} and 10^{th} day after vaccination and on 6^{th} day. It must be emphasized that the stimulative effect on the immune system of neuropeptides is different. Thus according to our previous trials some neuropeptides have a strong immunoprotective and a reserved immunostimulant activity, while others had quite an expressed immunostimulant, antibody synthesis stimulating activity. There is a reason to consider that with the aid of the studied neuropeptides it is possible to compensate the shortage of vaccine response by the potentiation them with the neuropeptides which shorten the duration of lag period of antibody synthesis.

Key words: *neuropeptides, mouse, vaccination response*

Introduction

Regardless of the intensive research work in neuroimmunology (Blalock, 1997; McCann et al., 1998), the cerebral cortex neuropeptides, especially in swine are poorly studied. Thus concerning the porcine brain cortex one is able to note only some works. Thus Kovaru et al. (1998) have studied the GTP-binding proteins of pig embryo cerebral cortex, which are connected with the lymphoid system. It is also investigated the connection of central nervous system (CNS) and immune system (IS) in stress (Hicks et al., 1998). Great interest provides the investigation of the molecular structure of substance P and related peptides (Nilsson, 1998; Nilsson et al., 1998).

The aim of this study was to elucidate if the porcine brain cortex neuropeptides have an immunostimulant activity, and if so, the possibility to compensate with neuropeptides the shortage of *Salmonella typhimurium* vaccine, i.e. to stimulate the antibody synthesis in the early phase of immune response.

Materials and Methods

Animals

Pigs

Adult healthy Estonian bacon breed swine were slaughtered in the slaughter-house and decapitated. The heads were transported within 1 hour into the laboratory.

Mice

Swiss white mice (both sexes) were reared up to the weight of 20–25 g and were housed in a room temperature 18–22 ºC.

Vaccine

Heat inactivated suspension (0.7×10^9 cells/ml) of *Salmonella typhimurium* strain 34–96 served as the vaccine.

Neuropeptide (NP) extraction

In laboratory the cranium was opened, the brain was placed into refrigerator (–20 ºC) for 2–3 hours and thereafter the cortex was prepared. The extraction of NP was performed as previously described (Karus et al., 1998). Shortly, cooled cerebral lobe cortex crown and parietal lobe dextra were separately chopped up in the presence of 1.5-fold volume of cold phosphate-buffer pH 7.4, and were ground up five minutes in a blendor on ice. Supernatants were separated by centrifuging the mixture by 8000 g for ten min. To the supernatants 2-fold volume of cold acetone was added. Produced precipitated extracts were dried and weighed. The main fractions were separated from the extracts. The chemical characterization of extracts and isolated peptides was done using spectrophotometrical studies (Shimadzu Ltd., UV-1601), IEF (Bio-Rad Ltd.), SDS-PAGE (Himifil Ltd.), etc. Peptide content was measured, and for further study the 1 μg/ml solutions of CCb (cerebral crown main fraction) and Pld (parietal lobe dextra main fraction) neuropeptides were made in above-described phosphate buffer. The working solutions with concentration about 140 ng/ml NPs were made before the animal trials. All injected solutions were sterilized.

Experimental design

Mice were simultaneously challenged on the first experimental day with 0.3 ml of vaccine intraperitoneally. NP solution in phosphate buffer pH 7.4 was injected subcutaneously, dose 66 ng (~3.6 ng/g) per mouse. Mice were bleeded through decapitation accordingly in experiment 1 (Table 1) on 1, 6, 9 and 21 day. In experiment 2 (Table 2) on 1, 3, 5, 7 and 10 day. The agglutinine titres of blood sera were assessed trivially and the – log was calculated.

Statistical analyze was done using SYSTAT.

Results

Neuropeptide CCb experienced a positive effect only on the 6th day of experiment (Table 1).

Table 1. Agglutinine titres (-log) in CCb challenged and vaccinated mice
Tabel 1. *Aglutiniinide tiiter (-log) CCb manustatud ja vaktsineeritud hiirtel*

Neuropeptide manipulation *Neuropeptiid*		1st day	6th day	9th day	21st day
None *Kontroll*	Mean *Keskmine*	0	1.24	1.48	1.48
n=20	StDev	0	0.13	0.16	0.16
CCb	Mean *Keskmine*	0	1.66	1.42	1.42
n=20	StDev	0	0.39	0.16	0.16

Remarks. Vaccine and CCb were injected simultaneously on the 1st day

In experiment 2 (Table 2) Pld possessed markedly stronger immunostimulatory effect in the early phase of antibody synthesis. Thus the influence of Pld was observed on 5, 7 and 10 day.

Table 2. Agglutinine titres (-log) in Pld challenged and vaccinated mice
Tabel 2*. Aglutiniinide tiiter (-log) Pld manustatud ja vaktsineeritud hiirtel*

Neuropeptide manipulation *Neuropeptiid*		1st day	3rd day	5th	7th day	10th
None *Kontroll*	Mean *Keskmine*	0	0.90	0.38	1.22	1.06
n=25	StDev	0	0.88	0.85	0.92	0.13
Pld	Mean *Keskmine*	0	0	0.96	1.91	1.96
n=25	StDev	0	0	1.04	0.83	0.65

Remarks. Vaccine and Pld were injected simultaneously on the 1st day

That means, that this type of immunostimulants as neuropeptides may have a curative significance in the practice as a supplemental remedy, especially in the case the extracellular pathogens.

Discussion

The data mentioned above indicate that NP, especially Pld had an immunostimulant activity, stimulating the early immune response (antibody synthesis). It is worth to remember that in the preliminary infection experiments CCb proved to be the strongest NP concerning its immunoprotective activity (Karus et al., 1998), in spite of now demonstrated low immunostimulatory activity, comparing with the effect of Pld. It is accepted that antimicrobial peptides effect the innate immunity (Boman, 1995; Zasloff, 1992). We have demonstrated earlier that the immunoprotective effect of NP (Fld) reveal mainly during the first five days after experimental infection with *S. typhimurium* (Karus et al., 1998, Kumar et al., 1999). But the results of this study demonstrate that NP influence positively also the first phase of adaptive immunity – the early antibody synthesis. Thus it may be possible to speak about therapeutic effect of vaccination. Our earlier findings (Kumar et al., 2000) affirm the position of Boman (1995) and Zasloff (1992) that antimicrobial peptides act on the innate immunity. However it is not yet clear which factor of innate immunity causes the stimulation of early antibody synthesis (Fearon, 1997; Fearon, Locksley, 1996). The existence of the connection between the innate or acquired immunity warranted by the function of macrophages or dendritic cells (Paluchka, Banchereau, 1999) or both, it has yet not to be answered. However, it may be possible that it has to do with other immunological factors. The newest experiments show (Yingwu Xu et al., 2000) that Toll-like receptors and some vectors are integral to both innate and adaptive immunity. Concerning the immunoprotective effect of Fld we have in our earlier studies (Kumar et al., 2000) found the protective index of Fld in mice during 1–5 days after the artificial infection was 0.24, while total protective index (1–10 days post infection) was only 0.05–0.13. Thus the main effect of Fld is limited with the first five days after the infection. Thus the neuropeptides influence mainly the innate immunity. As it was mentioned above, NP induces the antibody synthesis in its early period. Thus one may suppose that the potentiation of the vaccination (or vaccine) with immunostimulants like Fl may have a practical sense – namely in the prophylactics and treatment of young animals.

Conclusion

It is possible to potentiate the vaccination process with porcine cerebral cortex neuropeptides in the purpose of compensating the low rate of antibody synthesis in its lag phase, between 5–9 days.

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