[3, 10]. It must be pointed out that the antipolymerization activity of tuftsin is due mainly to the Pro-Arg sequence at the C-end.

The experiments thus showed that synthetic low-molecular-weight peptides can interact with different components of the blood clotting system. Heparin binding takes place invariably with the participation of free peptide amino groups, whereas an essential condition for peptide—fibrinogen interaction is the presence of free carboxyl groups. In both cases the dominant role is played by electrostatic forces, which is why the effects observed are weak.

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EFFECT OF PARACHLOROPHENYLALANINE AND 5-HYDROXYTRYPTOPHAN ON THE SERUM TRYPTOPHAN LEVEL AND ITS ABILITY TO STIMULATE TRYPTOPHAN UPTAKE BY CELLS

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KEY WORDS: tryptophan; blood serum; cell uptake.

According to data in the literature one of the factors determining the intensity of tryptophan metabolism in the brain and, in particular, along the pathway of serotonin synthesis, is the serum tryptophan level [3, 6, 10].

On the basis of indirect evidence it can be postulated that feedback (probably negative) exists between the intensity of serotonin biosynthesis in the brain tissue and the blood tryptophan level [4]. If such feedback exists, changes in the intensity of intracerebral serotonin metabolism induced experimentally ought to lead to a corresponding change in the blood tryptophan concentration. The investigation described below was undertaken in order to test this hypothesis experimentally.

As substances modifying the intensity of intracerebral serotonin metabolism, in this investigation it was decided to use parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, and the immediate precursor of serotonin — 5-hydroxytryptophan (5-HTP).

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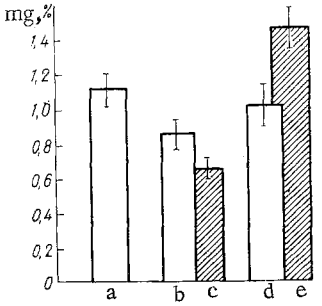

Fig. 1

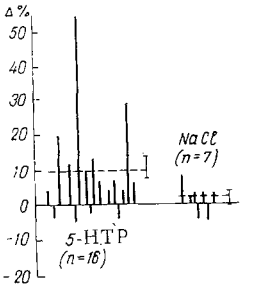

Fig. 2

Fig. 1. Effect of intraperitoneal injection of 5-HTP and PCPA on total serum tryptophan level in guinea pigs: a) before injection (n = 64); b) 2 h after injection of 0.9% NaCl (n = 12); c) 2 h after injection of 5-HTP (n = 18); d) 48 h after injection of 0.9% NaCl (n = 8); e) 48 h after injection of PCPA (n = 24).

Fig. 2. Effect of 5-HTP on activity of blood serum stimulating tryptophan uptake by cells. Ordinate, change in activity (in % of activity before injection). Vertical lines indicate individual values obtained in separate experiments. Broken line is mean statistical value.

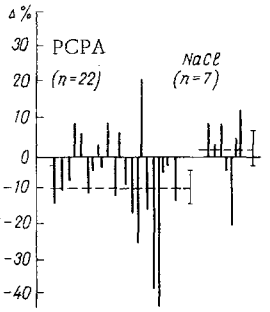

Fig. 3. Effect of PCPA on activity of blood serum stimulating tryptophan uptake by cells. Legend as to Fig. 2.

Simultaneously with determination of the blood tryptophan concentration, the ability of the blood serum of the experimental animals to stimulate tryptophan uptake by the cells was estimated. This ability, which according to data in the literature [7, 8] is due to the presence of a specific protein factor in the blood stream with electrophoretic mobility of γ-globulin, and whose biological role has not yet been explained, can provisionally be regarded as one of the components of the system regulating tryptophan metabolism in the intact organism.

EXPERIMENTAL METHOD

The methyl ester of PCPA (from Koch-Light, England) in a dose of 300 mg/kg, and 5-HTP (from "Biochemical Research," USA), in a dose of 200 mg/kg, were injected intraperitoneally in 1 ml of 0.9% NaCl solution into guinea pigs weighing 200-250 g, and the control animals were injected with 0.9% NaCl solution only. Blood was taken from the subclavian vein 1 h before injection of the compound and 48 and 2 h respectively after injection of PCPA and 5-HTP. Serum was separated by centrifugation for 10 min at 4°C and at 2500 rpm and kept until required for investigation at −20°C for not more than 3 days.

Tryptophan was determined quantitatively by a spectrofluorometric method [5]. Free tryptophan was separated from typtophan bound with serum proteins by ultrafiltration on "Centriflo" filters (from Amicon, USA).

The effect of serum on cell tryptophan uptake was determined on a model of chicken erythrocytes by the method described in detail previously [2], using [^{14}C]tryptophan (from Amersham Corporation, England).

EXPERIMENTAL RESULTS

The results of determination of the total serum tryptophan level of guinea pigs before and after injection of PCPA and 5-HTP are given in Fig. 1. The total tryptophan level was observed to fall 2 h after injection of 5-HTP from 1.12 ± 0.08 mg % (n = 64) to 0.66 ± 0.06 mg % (n = 18), whereas the fall in the blood tryptophan level of the control animals was significantly less marked. The difference between the blood tryptophan concentrations in the experimental and control animals (0.66 ± 0.06 and 0.86 ± 0.08 mg % respectively) reached the level of significance ($P < 0.05$).

The opposite picture was observed in animals receiving PCPA. In this case, 48 h after injection the serum tryptophan level increased to 1.46 ± 0.12 mg % (n = 24), significantly ($P < 0.05$) higher than the tryptophan concentration in the blood of the control animals (1.02 ± 0.11 mg %; n = 8).

Additional mathematical analysis by Student's test for tied pairs revealed a higher level of significance of the change in tryptophan concentration under the influence of both compounds ($P < 0.02$).

Determination of the concentration of free tryptophan, i.e., that not bound with plasma proteins, revealed similar changes in its level due to injection of PCPA and 5-HTP into the animals (these results are not shown).

The results of a study of the ability of serum to stimulate tryptophan uptake by chicken erythrocytes are given in Figs. 2 and 3. Injection of 5-HTP into the birds increased the uptake-stimulating activity of the blood serum by 10% (n = 16; $P < 0.05$ by Student's test for tied pairs), whereas in animals of the control group (n = 7) activity of the serum was unchanged. Injection of PCPA (Fig. 3), on the other hand, caused a significant decrease of 10% in the uptake-stimulating activity of the serum ($P < 0.01$ by Student's test for tied pairs).

Stimulation of serotonin synthesis by injection of 5-HTP thus lowers the blood tryptophan concentration, whereas a fall in the intensity of serotonin synthesis under the influence of PCPA raises the blood tryptophan level. Since the intensity of serotonin biosynthesis in the brain is determined by the blood tryptophan level [9-11], these results can be regarded as a possible argument in support of the existence of a control system aimed at maintaining the intensity of serotonin biosynthesis at a definite level.

One way of producing a rapid change in the blood tryptophan level may be by its uptake and subsequent metabolism by the peripheral tissues, especially liver cells, which under normal conditions metabolize more than 90% of all the tryptophan which enters the body [1].

It was accordingly decided to analyze correlation between the serum tryptophan level and its ability to stimulate uptake by the cells using a model of chicken erythrocytes. The presence of significant correlation between these parameters ($r = -0.467$; $P < 0.02$) suggests that a serum protein which, according to data in the literature [8], determines the ability of the serum to stimulate tryptophan uptake by cells, is a humoral factor in the system controlling the blood tryptophan level, and hence the intensity of serotonin synthesis in brain tissue. However, the central mechanisms regulating the activity of this serum factor so far remain completely unexplained; nevertheless, their elucidation is essential before a sufficiently complete scheme can be drawn up for the regulation of tryptophan metabolism *in vivo*.

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