THE EFFECTS OF TRYPsin-DEGRADATION PRODUCTS OF ALBUMIN ON THE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM

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Abstract. The investigations deal with the pharmacological activity of albumin degradation products. The peptides which resulted from 30 min trypsin digestion of human albumin exhibited some kinin-like effects. The peptides given intraperitoneally and intraventricularly revealed a central inhibitory action, the strength of which was similar to kinin effects.

INTRODUCTION

A considerable amount of data in the literature have shown that under multiple physiological and pathological states the activity of the body’s proteolytic enzymes increases (1, 5, 10, 12), and this leads to a greater amount of protein degradation products. The results of Buluk and Małofiejew (3), Małofiejew (15), Weyers et al. (17) and of Hamberg et al. (6) which have argued for a kinin potentiating action of the degradation products of certain blood proteins are suggestive of an important but underestimated role of these substances in the organism.

It was the aim of the present investigation to evaluate the effects of the degradation products of trypsin digested albumin upon the central nervous system.

MATERIAL AND METHODS

The experiments were carried out on 220 male Wistar rats (200–300 g) and on 70 male Porton-strain mice (20–25 g) fed a standard diet.

Trypsin degradation products of albumin (TDPA) were obtained from
human albumin (low salt content, produced by The Warsaw Serum and Vaccine Factory) digested with trypsin (The Warsaw Serum and Vaccine Factory). The conditions of the digestion process were as follows: 2.5% isotonic albumin solution in phosphate buffer (pH 7.4) was trypsinized in proportions of 2 mg per 100 mg of protein. The digestion was performed at 37°C for 30 min. The reaction was stopped after 30 min by heating the incubated mixture up to 56°C; the mixture was then dialysed against distilled water for 24 hr at 4°C, using a dialysing bag with pores of 100 Å diameter. The products derived by dialysis were concentrated; their amount per 1 ml was calculated as the quantity of nitrogen estimated according to Kjehdahl’s method (cit. accord. Homolka, 8).

As mentioned above TDPA obtained after a 30 min digestion were used. Bradykinin (Bradykinin triacetate, Reanal, Budapest) was injected into the lateral ventricle of the brain (ivc) as described by Herman (7).

The psychomotor activity of the animals was estimated with:
1. "Chimney" test (2). The mice were given ip TDPA in doses of 0.1, 1.0, and 10.0 mg/kg of the body weight. The time of the presence of mice in the chimney was measured at the 15th, 30th, and 60th min after injection of the substance studied.
2. "Open field" test (9). TDPA were injected into rats ip in doses of 0.4, 0.8, and 1.6 mg/kg. Psychomotor activity was measured 30 min after the injection.
3. Lát’s test (11). TDPA in doses of 1.6 mg/kg were given ip and ivc in the amount of 2.0, 4.0, and 10.0 µg per animal in a stable volume of 0.02 ml. Bradykinin in amounts of 2, 4 and 8 µg was given ivc. The preparations given ip were dissolved in physiological saline solution at a constant volume of 0.5 ml per a rat and 0.3 ml per a mouse. The control animals were given the same amounts of physiological saline. The preparations were administered in 0.02 ml amounts ivc to the animals Palaič’s artificial cerebro-spinal fluid was given in the same amount to the control group.

The results were elaborated statistically with the Student’s “t” test. The final results represent the average values from 8–9 estimations and include standard deviations (2 SD).

RESULTS

At the start of the experiments the efficacy of the proteolysis reaction in the mixture of albumin and trypsin had been established (Fig. 1) by determining the intensity of the release of non-protein nitrogen. It was found that the peptides derived from the 30th min digestion exerted optimum effects. TDPA thus obtained induced several kinin-like peri-
TRYPSIN-DEGRADATION PRODUCTS OF ALBUMIN

Fig. 1. Intensity of non-protein nitrogen formation during the process of trypsin digestion of albumin.

Peripheral effects, such as increased capillary permeability, a fall in the blood pressure, contraction of the smooth muscle layer of the isolated intestine (13). Moreover the activity of these peptides appeared to be about 30 times weaker than that of bradykinin when evaluated with the above parameters.

After the peripheral effects had been established it was decided to study the influence of TDPA on CNS.

"Chimney" test

TDPA given ip to mice caused a statistically significant prolongation of the mean ascent time, and these effects were in direct proportion to the dose and depended on the time of injection. The most effective dose appeared to be 10 mg/kg, which resulted in significant differences by the 15th min of the experiment when compared to the control. The same dose after 30 min doubled the ascent time, while at the 60th min there were effects (Fig. 2). Based on the above results in further experiments only the 30 min period after injection of TDPA was accepted.

"Open field" test

TDPA given at increasing doses revealed a psychodepressive action which manifested itself as a smaller number of rearings, episodes of interest in objects and crossed squares, and as a shortening of grooming time (Fig. 3). The above depressive effects were also related directly to the dose applied. The intermediate dose (0.8 mg/kg) of TDPA evoked
Fig. 2. The effects of trypsin digestion products of albumin (TDPA) on the behavior of mice evaluated with the "chimney" test.

Fig. 3. The behavior of rats in open field tested 30 min after TDPA injection.
significant differences in 3 out of 4 parameters evaluated. The highest dose (1.6 mg/kg) inhibited the psychomotor activity evaluated with all parameters.

**Lát's test**

Marked depressive effects after ip injected TDPA in the dose of 1.6 mg/kg were found in rats at the 15th, 30th, and 60th min of the experiment (Fig. 4). Peak activity was observed at the 15th min only.

![Figure 4](image)

*Fig. 4. Effect of TDPA given ip (1.6 mg/kg) on the behavior of rats in Lát's test.*

The behavioral changes could be due to the peripheral action of TDPA. In order to verify this suggestion the peptides were given ivc, and the effects of three different doses were evaluated after 15 min (Fig. 5). A direct relation was found between the psychomotor inhibitory action and the quantity administered.

The potency of the CNS psychomotor depressive action of TDPA was compared to the effects induced by ivc given bradykinin. As can be
Fig. 5. The behavior of rats injected ivc with 2.0, 4.0, and 8.0 μg of TDPA (Lát’s test).

seen in Fig. 6 bradykinin injected ivc in doses of 2μg and 4 μg revealed a symptomatic CNS inhibition which was similar in potency to the psychodepressive effects of TDPA.

Fig. 6. The behavior of rats given bradykinin in doses of 1.0, 2.0, and 4.0 μg as evaluated with Lát’s test.
DISCUSSION

The above results indicate that TDPA exhibit a definite biological activity which manifests itself in central effects, as well as the peripheral ones previously shown (blood pressure drop, greater capillary permeability, potentiation of bradykinin and histamine action, 13). Similar changes in the behavior of animals given ip TDPA, and at much lower ivc doses, suggest that they were due to the action of peptides on the same effector. However, the mechanisms by which TDPA act have not been completely elucidated.

Taking into account a similarity between the molecular mass of TDPA (13) and kinins (4) and the almost equal central effects after ivc administration to elicited by peptides and bradykinin, it is tempting to speculate that mechanisms by which both substances act are clearly related. One indication is that similar doses of bradykinin and TDPA produce similar central effects. Also, it has been demonstrated previously that bradykinin potentiates the action of certain analgesic drugs such as morphine and petidin. It also increases the psychodepressive and hypothermic effects of chlorpromazine and the analeptic action of psychedrin (16). Similar results were noted after trypsin-digested globulin peptides (14) and peptides derived from human albumin digested with leucocytes (19). Apart from the above effects, these peptides seem to induce a potentation of the CNS neurotransmitters. The results of our previous studies (18) have shown that the inhibitory central action of ACh can be enhanced with ivc given bradykinin. Similar results were obtained in the experiments with mice and rats, and they show the central inhibitory action of the peptides in various animals. The evidence accumulated thus far suggests that the effects of TDPA can be the consequence of their influence upon the central action of neurohormones.

The results discussed throw a new light upon the importance of the problem of endogenous protein degradation products as the substances of a high biological activity. For these reasons we can suppose that under the pathological conditions which proceed with an increased blood protein proteolysis the peptide degradation products formed can be the causal pathogenic factors.

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REFERENCES


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