THE EFFECT OF LEUKOCYTE-INDUCED DEGRADATION PRODUCTS OF ALBUMIN ON THE ANALGESIC ACTION OF MORPHINE

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Abstract. The effect of leukocyte-induced human albumin degradation products (LPAD) on the analgesic potency of morphine was investigated. The degradation products given alone to the rats did not affect pain threshold; while combined with morphine, they potentiated its analgesic action. Moreover, the degradation products increased the concentration of morphine in the brain tissue.

INTRODUCTION

A number of the body’s physiological and pathological states are followed by an increased activity of proteolytic enzymes (1, 5, 11). The enzymatic degradation of specific protein substrates results in known biologically active peptides, such as kinins, angiotensin, and fibrinogen-degradation products. Recent investigations from our Department and from other research centers (12, 13, 19, 23) have disclosed significant biological and pharmacological properties of trypsin products of blood protein degradation, too. Gibiński et al. (6–8) have found that leukocyte proteolytic enzymes show an activity towards plasma proteins in vitro.

The above observations encouraged the authors to perform a study on the possible pharmacological effects of peptides resulting from blood plasma proteins degraded by leukocyte proteolytic enzymes. The report presented here deals with leukocytic degradation products of human plasma albumin and their effect on the analgesic action of morphine.
MATERIAL AND METHODS

The experiments were carried out on 300 male Wistar rats weighing 250–300 g. The animals were fed a standard diet. They were kept at constant temperature and humidity. Before experiments, which were made at a similar time of day, rats were fasted for 24 h.

Leukocyte-induced degradation products of albumin (LPAD) were derived from human plasma albumin incubated with the leukocyte mass.

Blood samples were taken from the cubital vein in volunteers. The samples (1 vol of 3.8% sodium citrate: 5 vol of blood) were centrifuged at 1,000 rpm for 15 min at 4°C; plasma was decanted and leukocytes were transferred into glass tubes. After several washings with preserving fluid, the cells were concentrated to the final amount of about 100,000 per 1 mm³, counted in a Thoma–Zeiss counting chamber.

The digestion of 5% solution of human albumin (Serum and Vaccine Factory, Biomed) was performed in isotonic phosphate buffer (17.5 ml of 0.63 M NaOH + 82.5 ml of 0.164 M Na₂HPO₄) at pH 7.4, and 37°C. To 32 ml of albumin solution, four ml of chloroform were added and after exact shaking (denaturation of proteins) 4 ml of leukocyte suspension.

The digestion process was inhibited by heating the incubation mixture at 56°C for 15–20 min. The supernatant fluid (centrifugation at 10,000 rpm for 10 min, 4°C) in the amount of about 35 ml was put into a dialysing bag and dialysed for 20 h at 4°C against distilled water in the ratio 1:5. The concentration of peptides was estimated spectrophotometrically (apparatus type MOM 203) and their absorbance was read at wavelengths 260, 280, and 320 nm according to the formula:

\[(E_{280} - E_{320}) \times 1.45 - (E_{280} - E_{320}) \times 0.74\]

The duration of albumin digestion amounted to 0.5, 1, 2, 4, and 12 h.

The analgesic action of morphine was estimated on the basis of a changed threshold to pain induced by an electric stimulus. Morphine (Polfa) was injected intraperitoneally in a dose of 8 mg/kg. A biochemical method was used for quantitation of the levels of morphine in the brain tissue (24).

The process of digestion of albumin by leukocytes was assessed by means of the rate of release of non-protein nitrogen, according to the method of Kjeldahl (10).

For fractionation of LPAD (1 ml sample volume) Sephadex G-25 fine columns (1.9 cm × 50 cm) were applied. Fractions were collected at the rate of 2 ml/5 min. The content of peptides was estimated spectrophotometrically according to the formula given above.

The results, elaborated statistically with “t” Student’s test, were presented as averages of 6–8 estimations, including standard deviation (2 SD).
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RESULTS

Effects of digestion of albumin by leukocytic proteolytic enzymes evaluated with nonprotein nitrogen are illustrated in Fig. 1.

The highest increase in non-protein nitrogen occurred during the initial 60 min of incubation. Further incubation (4 h and longer) was without marked effect on the yield of released nitrogen. For this reason peptides derived from 30-min, 60-min and 120-min degradation products were chosen for studies on the analgesic action of morphine (Fig. 2).

Fig. 1. Increase in non-protein nitrogen as the result of albumin digestion by leukocyte enzymes.

Morphine has been found to increase the pain threshold up to maximum 8.4 V at 60 min of the experiment. The LPAD alone did not affect the pain threshold. Morphine given with LPAD derived from a sample after 30 min digestion elevated the pain threshold to 10.5 V. The analgesic administered with peptides from LPAD samples digested for 60 min and 120 min brought about an increase in the pain threshold to 14.5 V.

Fig. 2. The effect of leukocyte-induced human plasma albumin degradation products (LPAD) on the analgesic action of morphine (Mph). LPAD derived from 30, 60 and 120 min digestion. Dose of LPAD — 0.25 mg/kg.
thus pointing to potentiating effects of the latter on the analgesic action of morphine.

Next, the effects of various doses of LPAD from a sample after 60-min digestion on the action of morphine were assayed (Fig. 3). As can be seen in Fig. 3, a statistically significant difference in the dose-response relationship was observed at the 30th min of experiment; at the highest dose of the peptides (1 mg/kg), this relationship was visible by the 15th min.

The effect of peptides from LPAD sample after 60 min digestion (LPAD — 60 min) on the level of morphine in the brain tissue is presented in Fig. 4. The peptides from LPAD — 60' sample given in a dose of 1 mg/kg increased the concentration of morphine in the brain tissue by about 40% as compared to control animals given the drug alone.

Preliminary separation and purification of LPAD — 60' sample were made on columns packed with Sephadex G-25 fine gel (Fig. 5). It was shown that the peptides applied to the chromatographic columns yielded two distinct fractions No. 9 and 20; the approximate weight of the former was 4,200, and of the latter, about 1,200.
The pharmacological activities of the two fractions were compared to those of the mixture of peptides (Fig. 6). It was found that both fractions increased significantly the analgesic action of morphine at its peak activity (60th min). Moreover, a tendency towards potentiating the effects of morphine after its application with the fractions of peptides at 15th and 30th min was observed. In this respect, the more effective fraction of peptides appeared to be that with the lower molecule weight.

The influence of various amounts of peptide fractions on the analgesic action of morphine has been illustrated in Fig. 7. For purposes of clarity only the effects of peptides at the peak activity of morphine, i.e., at the 60th min have been presented. The observed effects have been
noticed to be dependent on a dose of the peptided used. This potentiating effect was more visible with the peptides of fraction 20, which had a lower molecular weight, than with those of fraction 9. Noteworthy is the fact that potentiation of morphine activity exerted by the unpurified peptides was nearly the same.

![Bar chart showing the effect of different doses of LPAD (60 min digestion) and of their separated fractions on the analgesic action of morphine 60 min after its injection.](image)

**DISCUSSION**

The investigations have shown that leukocytic enzymes exhibit an ability to digest human albumin, and that the products (LPAD) of this degradation process and their purified fractions markedly potentiate the analgesic action of morphine in rats. LPAD also increased the concentration of morphine in the rat's brain tissue. The most active fraction of peptides (fraction 20) was found to have a molecular mass near that of other biologically active peptides (2–4).

Similar potentiating effects on another analgesic, dolantin, have been observed by Malinowska (14) in conditions of increased blood fibrinolytic activity. The results of this author indicate that during conditions of elevated blood levels of fibrinogen degradation products, a potentiation of the analgesic action of dolantin takes place. These products brought about an increase in the concentration of the drug in the brain tissue, too (2, 3, 14).

The present results point to important pharmacological properties of LPAD which in the case of potentiation of morphine can depend on central or peripheral effects. The latter are hyperemia of the peritoneum.
and increased penetration of morphine into the blood circulation; higher blood concentration of the free fraction of the analgesic; changes in the blood–brain barrier, which results in an increased brain penetration and higher brain concentration of morphine. The possibility of the occurrence of these events is supported by the recently discovered biological and pharmacological properties of other substances of peptide structure. It has been shown that some of these peptides increase vascular permeability (17) and potentiate the action of several exogenous substances (15, 16, 21). The results of the present report, namely, the increase in morphine levels in the brain tissue on injection with LPAD are an example of the latter effect.

The central effects of LPAD peptides regarding morphine can be related to their influence on neurotransmitters of the CNS as in the case of kinins (20, 22). It has been reported by Schönhöfer (18) that kinin-like substances alter the intracellular level of cyclic 3' 5' AMP. One can suppose that the mechanism of central action of the peptides investigated by us is similar.

Although the above results do not allow one to establish unequivocally the exact mechanism of the action of LPAD, they point to the fact that, as with tissue hormones, the products of albumin degradation are active pharmacological substances which essentially alter the potency of several exogenous substances.

This investigation was supported by Project 09.4.1 of the Polish Academy of Sciences.

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Accepted 6 February 1976

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