PARADOXICAL RELATIONS BETWEEN EFFICACY OF INTRANASAL AND INTRAPERITONEAL ADMINISTRATION OF DERMORPHINS TO RATS

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In 1981 peptides, called dermorphins, were first isolated from the skin of frogs of the genus *Phyllomedusa*. The name "dermorphin" is nowadays used most frequently for the heptapeptide IAFGYPS-NH_2. Dermorphin-like peptides have now been found in the brain and stomach of mammals [7]. The analgesic activity of demorphins is about 10 times stronger than that of morphine and enkephalin [3, 4]. By now more than 200 analogs of dermorphins have been synthesized, but the dependence of their effects on structure, dose, and mode and time of administration remains largely unexplained.

The most widespread methods of administration of endogenous opioids are by the intravenous and subcutaneous routes. The efficacy of many drugs, if given by the intranasal route, which is clinically more convenient, is usually greatly reduced. However, there are indications that in certain cases peptides, administered this way, exhibit sufficiently high biological activity.

Considering the complexity of the dose-effect relationship for neuropeptides, it was decided to study dermorphin and its A-2 analog (IdAFdAYPS-NH_2) using two methods of administration: intraperitoneal and intranasal.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g. Dermorphin and its analogs were synthesized by the classical method in solution [5]. Peptides were administered in aqueous solution: intraperitoneally in a volume of 200 µl and intranasally in a volume of 20 µl. Control animals received distilled water. Analgesic activity was determined by the tail flick method immediately after administration of the preparation, and again 3, 6, and 24 h later. A separate group of animals was used for each time. At each period of investigation the nociceptive sensitivity was measured for 1 h with intervals of 10 min. Analgesic activity (A) was calculated by the equation:

$$A = \frac{t_0 - t_K}{30 - t_K} \cdot 100$$

where t_0 is the latent period of avoidance of the nociceptive stimulus by the experimental animals, t_K the latent period of avoidance of the nociceptive stimulus by the control animals, 30 the longest time of presentation of the nociceptive stimulus (in sec). To determine the mean value of analgesia, the value of A was averaged from the 20th to the 60th minute after the beginning of recording, during the period when the greatest analgesic effect was observed. The results were subjected to statistical analysis using Fisher's exact test and the chi-square test [1].

EXPERIMENTAL RESULTS

Considerable analgesia was observed following intraperitoneal injection of the peptide in a dose of 0.03 mg/kg, but a further increase of the dose to 0.1-1.0 mg/kg gave no analgesic effect, Only starting from 5 mg/kg did dermorphin induce considerable analgesia once more (Fig. 1).

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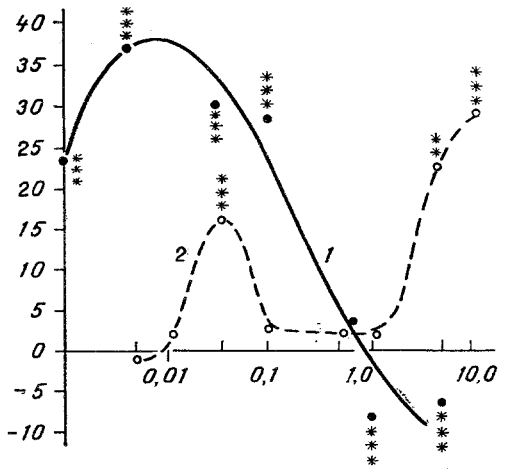

Fig. 1. Dependence of analgesic activity of dermorphin on dose when administered by intranasal (1) and intraperitoneal (2) routes. Abscissa, dose (in mg/kg) on a logarithmic scale; ordinate, degree of analgesia (in % of control). **p < 0.01, ***p < 0.005.

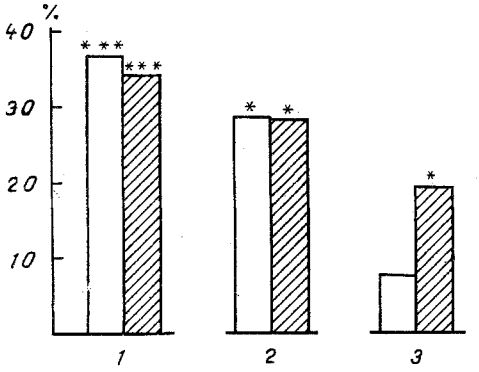

Fig. 2. Mean value of analgesic activity at different times after intranasal injection of 0.005 mg/kg of dermorphin (unshaded columns) or A-2 analog (shaded columns). Ordinate, degree of analgesia (in % of control). 1) During 1 h after administration; 2) 3 h, 3) 6 h after administration. *p < 0.01, ***p < 0.005.

The A-2 analog, when injected intraperitoneally, exhibited activity similar to that of dermorphin.

When the intranasal route was used analgesia was observed after very small doses of dermorphin — from 0.001 to 0.1 mg/kg. An increase of the dose to 1 and 5 mg/kg led not only to disappearance of the effect, but actually to an increase in nociceptive sensitivity (Fig. 1).

To study the duration of the effect, a dose of 5 mg/kg was given intraperitoneally and a dose of 0.005 mg/kg by the intranasal route, i.e., doses inducing a marked analgesic effect. The duration of the analgesic activity of the preparations when given intraperitoneally was 60-90 min for both peptides.

The maximal analgesic effect when dermorphin and A-2 were given by the intranasal route in a dose of 0.005 mg/kg was observed in the course of 1 h after injection: A amounted to 38 and 35%, respectively (Fig. 2). After 3 h the analgesic activity of both preparations showed a small decrease, but after 6 h only A-2 still preserved its activity.

The results are thus evidence, first, of the paradoxical character of the dependence of the analgesic activity of dermorphin on dose when given intraperitoneally. The two peaks of analgesic activity on the dose-effect curve in the presence of an intermediate zone with a certain degree of hyperalgesic action create an extremely complex situation. Second, a strong and prolonged analgesic activity of unusually small quantities of dermorphin and its A-2 analog was found when given by the intranasal route.

The only possibility of an explanation, in principle, of the unusual character of the dependence on dose in the case of intraperitoneal injection must evidently be to accept the coexistence of different mechanisms of the onset of analgesia. At present it is only possible to cite information on the presence of regions in the brain that respond differently to small and large doses of dermorphin [8]. It can be tentatively suggested that dermorphin, acting on receptor systems of the body differing in localization and sensitivity, leads to a complex resultant physiological effect.

So far as the extremely high efficacy of intranasal administration is concerned, the majority of sources in the literature describe a relatively high rate of degradation and considerable losses of biologically active peptides when given by the intranasal route. It has been shown, for example, that only 10% of Leu-enkephalin, administered by this method, enters the blood stream [9], and that the biological activity of follicle-stimulating hormone is only 1-6% of that when given intravenously [10]. At the same time, there are reports of absorption of a large fraction of enkephalin analogs in the nasopharynx — up to 70% of the quantity entering the blood stream when given intraperitoneally [6]. However, the absorption and distribution of dermorphin in the body may evidently be different in principle from that of other endogenous neuropeptides, for it contains an alanine enantiomorph which stabilizes it.

What is the possible mechanism of such a powerful antinociceptive effect of dermorphin when given intranasally? The first alternative is that the peptide can penetrate from the nasopharyngeal mucosa into lymphatics, which are anatomically connected with the lymphatic system of the hypothalamus [2]. In that way even small quantities of peptides can be "supplied" directly to the brain structures, bypassing the blood stream. The second alternative mechanism of the onset of analgesia after intranasal administration is the reflex action of the peptide through numerous receptors in the mucosa of the nasal cavity, nasopharynx, and pharynx. This hypothesis has many objections. In particular, the quite long duration of the effect and the closeness of the median effective doses for intranasal administration and doses in the region of the "minor" peak of analgesic activity following intraperitoneal injection, are difficult to reconcile with it.

The data given above are evidence of the existence of more complex mechanisms of action of certain peptides than was hitherto imagined. They must be taken into account not only from the theoretical aspect, but also in research aimed at subsequent clinical application of these compounds.

Considering the very low level of effective doses of dermorphin when given intranasally, and also the duration of the analgesia thus induced, there is good reason to investigate to what extent the side effects of dermorphin, which prevent its clinical use, are preserved if it is administered by this route.

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