by comparison with the retention times of the pure standards.

Suitable quantities of the cell effluent, collected corresponding to the chromatographic peaks of the 2 drugs under examination, were tested for homogeneity by qualitative bidimensional TLC using Merck silica-gel precoated glass plates (20 × 20 cm, 0.25 mm).

The 1st development used (12 cm) was benzene : dioxane : ethanol : concentrated ammonia (50:40:5:5, v/v) and the 2nd was ethanol saturated with sodium chloride : acetic acid : water (70:20:5, v/v)[11]. After double development, the plates were dried for 25 min at 110 °C and sprayed, in the order, with diazoted p-nitroaniline and concentrated hydrochloric acid. The chromatogram obtained in this way corresponded perfectly in colour (green) and in R_f-values to that obtained developing in the same manner the pure reference compounds.

Figure 2 shows, as an example, typical liquid chromatograms of brain samples of untreated and imipramine treated rats, and the table reports the mean plasma and tissue concentrations of imipramine and of its metabolite detected at various times after i.p. administration of 50 mg/kg of imipramine.

In the case of plasma 5 ml were used for the extraction and concentrated to 50 µl; considering that the amount we were able to detect was 15 ng in column for both substances (figure 1), the lower sensitivity limit is about 20 µg l^{-1}. However, by using a smaller microcell than that at our disposal, the sensitivity can be further raised by about 5–8 times.

We can therefore conclude that our method is rapid, easy to perform, specific and allows the simultaneous dosage of both imipramine and desipramine; moreover this technique presents a good sensitivity, comparable, in the conditions we adopted, to that obtained by Weder and Bickel[8] by the gas chromatographic assay.

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Effect of the administration of (d-Ala) ^{2}methionine-enkephalin on the serotonin metabolism in rat brain[1]

A. Algeri, A. Consolazione, G. Calderini, G. Achilli, E. Puche Canas[2] and S. Garattini

Istituto di Richerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milano, (Italy), 8 March 1978

Summary. The effect of cerebroventricular injection of [D-Alanine] methionine-enkephalin (DALA), a synthetic analog of met-enkephalin, on the serotonergic system of rat brain has been studied. This opioid peptide caused an increase in 5HT turnover which was particularly evident in the limbic forebrain. This effect was completely antagonized by naloxone pretreatment.

It is now established that intracerebral injection of methionine-enkephalin or leucine-enkephalin, 2 pentapetides extracted from mammalian brain[3,4], gives rise in the rat to symptomatology, such as analgesic and decreased motor activity, closely resembling that elicited by morphine and other narcotic drugs[5,6].

Due to their rapid inactivation by brain endopeptidases[7,8], these peptides have a very short-lasting effect, and it is not possible by the usual methodological approaches to demonstrate modifications in the metabolism of central monoamines of the type correlated with the analgesic activity of morphine[9].

We have recently reported that (D-Ala) ^{2}methionine-enkephalin (DALA), a synthetic analog of methionine-enkephalin made resistant to enzymatic cleavage by substituting D-alanine for glycine in the second position of the peptidic chain[10], gives not only a more powerful pharmacological action, but also a clear stimulant effect on the synthesis of brain dopamine[11,12].

There is evidence that the central serotonergic system is involved in the analgesic effect of morphine in the rat, and administration of this drug is known to increase serotonin (5HT) turnover[9,13]. The objective of the experiments reported here was to determine whether the similarity between morphine and DALA, as regards the pharmacological action and the effect on central dopaminergic system[11,12], also existed as regards the effect on brain 5HT.

DALA was injected into unanaesthetized rats (175–200 g male Sprague-Dawley, Charles River, Calco, Italy) through 2 indwelling polyethylene cannulas implanted in the lateral ventricles 3 days before the experiment. The peptide was dissolved in saline and injected in the volume of 5 µl/ventricle at different concentrations and at different times before killing. The brains were rapidly excised, dissected, frozen, and kept frozen until the assays. Serotonin, its metabolite, 5-hydroxyindolacetic actid (5HIAA) and its precursor tryptophan (TP) were determined fluorimetrically in the whole brain, purified in organic solvent according to the procedure of Curzon and Green[14] and Denkla and Dewey[14,15]. Intraventricular injection of 25 µg of DALA, a dose which induced analgesia, immobility and increased dopamine turnover[6] led to an increase of brain 5HIAA (experiment I). Levels were significantly raised 45 min after the injection, and increased further to 90 min, when they peaked out. After 135 min, 5HIAA levels were still high. A lower dose of the peptide (10 µg) led to an increase of 5HIAA, but this did not reach the level of a statistical significance (experiment II). A higher dose (50 µg), on the other hand, was no more effective than 25 µg (experiment II). None of these experimental conditions changed the brain concentration of 5HT (data not reported).

The DALA-induced rise in 5HIAA appears due more to an increased rate of formation of this metabolite than to DALA-induced inhibition of its efflux from the brain. In

Effect of DALA administration on 5HIAA and TP in the concentrations in rat brain

			min after DALA		
		Controls	45	90	135
Experiment I	5HIAA µg/g ± SE	0.42 ± 0.03	0.55 ± 0.03[a]	0.66 ± 0.02[a]	0.66 ± 0.02[a]
			Dose of DALA (µg/rat)		
		Controls	10	25	50
Experiment II	5HIAA µg/g ± SE	0.57±0.02	0.63±0.02	0.69±0.03[b]	0.70 ± 0.03[b]
	TP µg/g ± SE	5.2 ±0.2	5.3 ±0.3	5.4 ±0.3	n.d.[c]

DALA, dissolved in 10 µl of saline was administered in the lateral ventricles. Controls were injected with saline only. Experiment I: Rats were killed at different intervals after administration of 25 µg of DALA into the lateral ventricles. [a]Difference from 0 time $p < 0.01$ with Duncan's new multiple test. Experiment II: Rats were killed 90 min after injection of different doses of DALA into the lateral ventricles. [b]Difference from control $p < 0.05$ with Duncan's new multiple test. [c]n.d. = not determined. Values are the mean ± SE of 5 samples.

fact, when the efflux is blocked by administration of probenecid (200 mg/kg i.p.)[16] 5HIAA accumulates in the limbic forebrain, an area rich in serotonergic nerve terminals, to a significantly greater extent in animals treated with the peptide than in those receiving the vehicle only (figure 1). As neither the levels of 5HT nor the transport of its acidic metabolite are affected by DALA, we can tentatively conclude that the opioid peptide acts on the serotonergic systems by increasing synthesis of this neurotransmitter.

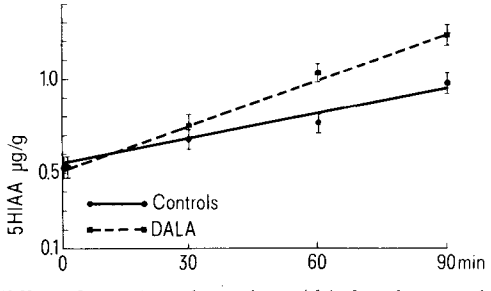

Fig. 1. Effect of DALA on the probenecid-induced accumulation of 5HIAA in rat limbic forebrain. DALA (25 µg/rat) was injected into the lateral ventricles 5 min after probenecid (200 mg/kg i.p.) and 5HIAA was determined 0, 30, 60 and 90 min after probenecid injection. Control rats received probenecid + DALA vehicle (saline). Each point is the mean±SE of 5 observations. Regression lines were constructed by the least squares method. The lines were not parallel ($p < 0.01$) and were significantly different ($p < 0.01$). The regression coefficients were 8.16 for the DALA group and 4.56 for controls ($p < 0.05$).

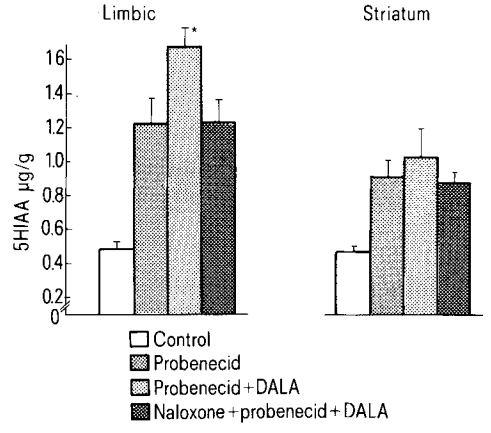

Fig. 2. Effect of naloxone pretreatment on probenecid-induced accumulation of 5HIAA in rat limbic forebrain and striatum. Naloxone (2 mg/kg i.p.) was administered 15 min before and probenecid (200 mg/kg i.p.) 5 min before DALA (25 µg/rat). 5HIAA levels were determined 90 min after probenecid.

Similar results were obtained by Goodlet and Sugrue[13] with morphine, but not with other opiates such as pentazocine, methadone and pethidine. The action of DALA on brain 5HT is therefore closer to that of morphine. However, morphine increases the concentration of tryptophan[13], whereas DALA does not (table). This finding enables us to exclude the possibility of the enhancement of 5HT synthesis being due to increased precursor availability.

The ability of DALA to increase probenecid induced accumulation of 5HIAA in the limbic forebrain is completely antagonized by pretreatment with an opiate antagonist such as naloxone (figure 2). This antagonism suggests that in the serotonergic system DALA interacts with the opiate receptors. Probenecid induced accumulation of 5HIAA in striatum is not affected by DALA treatment. This peptide thus appears to modulate serotonergic neurons only in specific serotonergic regions. Much experimental evidence indicates the similarity between the properties of DALA and naturally occurring enkephalins[10]. We can therefore infer that the effect seen on the 5HT system after treatment with DALA is a characteristic of opioid peptides.

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