York, 1972) vol. 7, pt. 1, p. 245; P. A. Liebman, in *ibid.*, p. 481; W. R. A. Muntz, in *ibid.*, p. 529; and Stell and Hárosi (*6*). We have recently examined turtle cone oil droplets and found no trace of a pigment similar to that of ellipsosomes even in the colorless (ultraviolet absorbing) droplets.
6. W. K. Stell and F. I. Hárosi, *Vision Res.* **16**, 647 (1976).
7. The calcium-free and complete saline solutions contained the following:

Chemical	Concentration (m*M*)	
	Calcium-free	Complete
NaCl	133.5	100.0
$NaHCO_3$	11.9	5.0
NaH_2PO_4	3.3	0.5
KCl	3.4	2.5
$MgCl_2$	21.0	1.0
$CaCl_2$	0	1.5
Glucose	11.1	10.0
Sucrose	0	90.0
Adjusted *p*H	7.4	7.0

In all these experiments 10 m*M* $MgCl_2$ was inadvertently used in the complete medium without apparent ill effects.
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10. Frequencies and wavelength of α, β, and γ peaks were as follows:

Source	Frequency	Wavelength (nm)	Source
	α Peak		
Xipophorus	545.9	549.6	Ellipsosomes
Poecilia reticulata	546.2	549.2	Ellipsosomes
Fundulus	546.0	549.0	Ellipsosomes
Poecilia reticulata	521.5	575.2	Erythrocytes
Reduced cytochrome c	545.5	550.0	Mitochondria
	β Peak		
Xipophorus	575.6	521.2	Ellipsosomes
Poecilia reticulata	576.5	520.4	Ellipsosomes
Fundulus	575.0	522.0	Ellipsosomes
Poecilia reticulata	555.8	540.0	Erythrocytes
Reduced cytochrome c	576.0	521.0	Mitochondria
	γ Peak		
Xipophorus	720.7	416.3	Ellipsosomes
Poecilia reticulata	721.6	415.7	Ellipsosomes
Fundulus	720.0	417.0	Ellipsosomes
Poecilia reticulata	724.0	414.0	Erythrocytes
Reduced cytochrome c	723.0	415.0	Mitochrondria

The data for mitochondria are from H. R. Mahler and E. H. Cordes, *Biological Chemistry* (Harper & Row, New York, ed. 1, 1966), table 14–10. In this table the extinction per millimole is given for the γ band as 125. The average peak density of *Xiphophorus* cones was more than 0.7, indicating a concentration of more than 11 m*M*.
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19 December 1977; revised 24 February 1978

Stimulation of Dopamine Synthesis in Caudate Nucleus by Intrastriatal Enkephalins and Antagonism by Naloxone

Abstract. *The intraventricular injection of methionine-enkephalin (50 to 100 micrograms) or [D-Ala²]-methionine-enkephalinamide (1.5 to 12 micrograms), a synthetic enkephalin analog resistant to enzyme degradation, caused a marked dose-dependent increase in dihydroxyphenylacetic acid and homovanillic acid concentrations in the rat striatum. The [D-Ala²] analog increased the accumulation of dopa in the striatum after aromatic amino acid decarboxylase inhibition, indicating that it increased dopamine synthesis. At the highest doses used both enkephalins failed to modify brain serotonin metabolism. The monolateral microinjection of the [D-Ala²] analog (3 to 6 micrograms) into the caudate nucleus increased the concentration of dihydroxyphenylacetic acid in the injected side, whereas bilateral injection increased the concentration of this compound in both caudate nuclei and caused catalepsy. The stimulant effect of the [D-Ala²] analog on dopamine synthesis in the striatum persisted after destruction of striatal postsynaptic dopamine receptors with kainic acid. The biochemical and behavioral effects of enkephalins were prevented by naloxone, a specific narcotic antagonist. The results indicate that enkephalins stimulate dopamine synthesis by an action on opioid receptors localized on dopaminergic nerve terminals.*

The central effects of morphine and opiate-like drugs are considered to be mediated by specific opiate receptors that have been mapped in the mammalian brain (*1*). Endogenous peptides with opiate agonist properties have been isolated from the mammalian brain and identified as the pentapeptides methionine-enkephalin and leucine-enkephalin (*2*).

However, the two enkephalins exert a weak morphine-like effect when injected intraventricularly or intracerebrally into rats because they are rapidly destroyed by peptidases in brain (*3*). An enkephalin analog, [D-Ala²]methionine-enkephalinamide, has been synthesized which has almost the same affinity for the opiate receptors as the endogenous peptides but is resistant to enzymatic degradation (*4*). Thus the [D-Ala²] analog induces profound and long-lasting analgesia and other opiate-like behavioral effects when injected intracerebrally (*4*), and therefore might be useful in the study of the mechanism of action of endogenous and exogenous opiates. Narcotic analgesics stimulate dopamine turnover (*5*, *5a*), but the mechanism of this effect is not clear. Yet this action has been correlated with several central effects of these compounds, such as the analgesic effect, catalepsy, motor stimulation, circling behavior, and tolerance (*6*).

We now report that methionine-enkephalin and, to a much greater degree, the [D-Ala²] analog stimulate striatal dopamine synthesis when injected intracerebrally. This effect is reversed by naloxone, a specific opiate antagonist, but persists after destruction of striatal dopamine receptors with kainic acid, suggesting that enkephalins stimulate dopamine synthesis by an action on opioid receptors localized on dopamine nerve terminals.

Male Sprague-Dawley rats (260 to 280 g) were implanted stereotaxically with

Table 1. Effect of the intraventricular injection of methionine-enkephalin and the (D-Ala²) analog on dopamine metabolism in the caudate nucleus. The [D-Ala²] analog and methionine-enkephalin were given intraventricularly 45 and 15 minutes, respectively, before the animals were killed. Naloxone (3 mg/kg, intraperitoneally) was given at the same time as the peptides. Each value is the mean ± standard error of six determinations. Plus and minus signs indicate degree of catalepsy.

Treatment	Amount (μg)	Dopamine (μg/g)	DOPAC (μg/g)	HVA (μg/g)	Catalepsy
Saline		10.61 ± 0.51	2.36 ± 0.01	1.73 ± 0.9	−
Methionine-enkephalin	50.0	10.05 ± 0.60	2.75 ± 0.01*	2.21 ± 0.01*	−
Methionine-enkephalin	100.0	12.85 ± 0.39*	2.96 ± 0.01†	2.75 ± 0.02†	+
[D-Ala²] analog	1.5	10.11 ± 0.46	2.77 ± 0.02†	2.45 ± 0.01†	−
[D-Ala²] analog	3.0	10.08 ± 0.51	3.02 ± 0.01†	2.75 ± 0.01†	++
[D-Ala²] analog	6.0	12.41 ± 0.38*	3.97 ± 0.01†	3.11 ± 0.02†	+++
[D-Ala²] analog	12.0	13.31 ± 0.61*	4.34 ± 0.02†	3.61 ± 0.01†	+++
Naloxone			2.32 ± 0.01	1.65 ± 0.09	−
Naloxone plus methionine-enkephalin	100.0		2.33 ± 0.02	1.90 ± 0.01	−
Naloxone plus [D-Ala²] analog	3.0		2.38 ± 0.02	1.88 ± 0.01	−

*$P < .01$ with respect to saline-injected rats. †$P < .001$ with respect to saline-injected rats.

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permanent 23-gauge stainless steel guide cannulas aimed for the lateral ventricle on one side, or for the head of the caudate nucleus, and terminating 2 mm dorsal to the area designated by coordinates AP 2.3, LAT 2.8, DV 5, of Pellegrino and Cushman (*7*). Drugs were injected into the caudate nucleus through a 30-gauge microsyringe calibrated to extend precisely 2 mm beyond the tip of the guide cannula, while the tip of the microsyringe for the lateral ventricle did not protrude past the guide tip.

All injections were given at least 5 days after surgery to unanesthetized animals. For the caudate nucleus, the rate of injection was 0.1 μl for 30 seconds and the injection volume was 0.5 to 1.0 μl. Volumes of 2.5 to 5 μl were injected into the lateral ventricles in 2.5 to 5 minutes.

Placements in the lateral ventricle were assessed by withdrawal of spinal fluid. Standard histological verification of injection sites, made in two animals randomly taken from each experimental group, revealed that the placements aimed for the caudate nucleus were correct. Dopamine (*8*), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) (*9*), serotonin and 5-hidroxyindoleacetic acid (*10*) were assayed fluorometrically. The L-dopa was assayed as described by Kehr *et al.* (*11*). Catalepsy (akinesia) was estimated as described by Costall and Naylor (*12*).

Methionine-enkephalin and the [D-Ala^2] analog were synthesized by one of us (C.D.B.) by the solid phase method. 3-Hydroxybenzylhydrazine hydrochloride (NSD 1015) was from Hoffman-La Roche.

Methionine-enkephalin was dissolved in Ringer solution. Control injections for this enkephalin consisted of vehicle alone adjusted to *p*H 4.0 to approximate that of the drug solution. The [D-Ala^2] analog was dissolved in twice-distilled water and the control for this compound was saline. Comparison between groups was calculated by one-way analysis of variance followed by Student's *t*-test. Comparisons between peptide- and saline-injected sides were made by one-way analysis of variance of individual differences between the two sides.

The intraventricular injection of the [D-Ala^2] analog (1.5 to 12 μg) induced a dose-dependent increase in the concentration of HVA and DOPAC in the caudate nucleus (Table 1). Much higher doses of methionine-enkephalin (50 and 100 μg) were necessary to increase the dopamine metabolites.

The highest doses of these enkephalins caused a modest but significant increase in the concentrations of dopamine in the caudate nucleus but failed to modify the concentrations of serotonin and 5-hydroxyindoleacetic acid in the whole brain.

To determine whether the changes in the concentrations of the dopamine metabolites reflected an increased rate of dopamine synthesis we studied the formation of L-dopa after inhibition of the aromatic aminoacid decarboxylase with NSD 1015 (*13*). The results are shown in Table 2. As expected (*14*), rats treated with 6 μg or more of the [D-Ala^2] analog exhibited catalepsy (akinesia) that lasted for more than 1 hour.

Table 2. Effect of intraventricular injection of the [D-Ala^2] analog on dopa accumulation (per 30 minutes) in the caudate nucleus after inhibition of aromatic amino acid decarboxylase with NSD 1015. The NSD 1015 (100 mg/kg) was injected intraperitoneally 15 minutes after the administration of the [D-Ala^2] analog. The latter, or saline, was injected intraventricularly 45 minutes before the animals were killed. Naloxone was given as reported in Table 1. Each value is the mean ± standard error of ten determinations.

Treatment	Amount (μg)	Dopa (ng/g)
Saline		750 ± 36
[D-Ala^2] analog	3.0	1060 ± 58*
[D-Ala^2] analog	6.0	1250 ± 68*
Naloxone		690 ± 70
Naloxone plus analog	3.0	820 ± 45†

*$P < .01$ with respect to saline treatment. †$P < .01$ with respect to treatment with the [D-Ala^2] analog (3 μg).

Table 3. Effect of intrastriatal injection of the [D-Ala^2] analog on the concentration of DOPAC and on dopa accumulation in intact and kainic acid–lesioned caudate nucleus. The analog or saline was injected into normal and kainic acid–lesioned caudate nuclei of one side. Saline was injected in the contralateral caudate nucleus. Kainic acid (2 μg in a volume of 1 μl) was injected (in a period of 5 minutes) 10 days beforehand into the right caudate nucleus of anesthetized (Equithesin) rats. As reported (*20*), the injection of kainic acid resulted in DOPAC concentrations that were about 60 percent higher in the lesioned side than in the contralateral side; moreover, the basal activity of dopamine-sensitive adenylate cyclase was reduced by 40 percent and the enzyme was completely insensitive to dopamine stimulation. Dopa accumulation was studied in animals treated with NSD 1015 according to the schedule reported in Table 2. Each value is the mean ± standard error of at least four experiments.

[D-Ala^2] analog (μg)	Percentage of contralateral saline-injected side	
	DOPAC	Dopa accumulation
Normal caudate nucleus		
Saline	108 ± 5	96 ± 13
3.0	175 ± 8*	168 ± 15*
6.0	210 ± 15*	196 ± 20*
Kainic-lesioned caudate nucleus		
Saline	160 ± 13*	118 ± 14
3.0	232 ± 21†	153 ± 16†
6.0	320 ± 18†	191 ± 21†

*$P < .01$ with respect to contralateral caudate nucleus. †$P < .01$ with respect to the homolateral caudate of rats injected with saline.

Naloxone, a specific blocking agent of opiate receptors, at the dose of 3 mg/kg, prevented not only the behavioral effects of the peptides, in agreement with previous results (*4*), but also their effect on dopamine metabolism (Tables 1 and 2), showing that this effect is mediated by opiate receptors.

In an attempt to determine the site of action of enkephalins in stimulating dopamine turnover, we injected the [D-Ala^2] analog into the caudate nucleus of one side and the vehicle into the contralateral side. As shown in Table 3, the analog produced a dose-related increase in the concentration of DOPAC and enhanced dopa formation after treatment with NSD 1015 in the injected side. Bilateral microinjections of the analog (3 μg in each caudate nucleus) increased the DOPAC concentration by about 70 percent in both sides and produced catalepsy (results not shown).

It has been suggested that morphine and other narcotic analgesics accelerate dopamine turnover by a neuroleptic-like mechanism [see (*5, 5a*)]. According to this theory, the increase in dopamine turnover would be a compensatory response to the blockade of striatal dopamine receptors, with catalepsy as a pharmacological correlate.

To determine whether postsynaptic dopamine receptors in the caudate nucleus were involved in the effect of enkephalins on dopamine synthesis, we studied the effects of the [D-Ala^2] analog on dopamine synthesis in a caudate nucleus lesioned with kainic acid which destroys striatal nerve cell bodies but leaves dopaminergic terminals intact (*15*). The results shown in Table 3 indicate that the analog still increased DOPAC concentrations and enhanced L-dopa formation after decarboxylase inhibition.

Methionine-enkephalin and the [D-Ala^2] analog share with morphine and other narcotic analgesics the property of stimulating dopamine synthesis. This effect is readily antagonized by naloxone, indicating that it is mediated by an action on narcotic receptors.

The site of this action of enkephalins is likely to be the caudate nucleus, because the effect can be produced after local injection and is confined to the injected side. The finding that the [D-Ala^2] analog maintains its capacity to increase dopa-

mine synthesis in caudate nuclei lesioned with kainic acid indicates that neither postsynaptic dopamine receptors nor the integrity of a striato-nigral feedback loop are essential for this action of enkephalins.

Since, in the rat, the caudate nucleus contains the largest number of opiate receptors (*1*), the opiate receptors are associated with synaptosomes (*16*), and lesion of the nigro-striatal dopaminergic pathway with 6-hydroxy-dopamine eliminates the high-affinity binding for dihydromorphine in the caudate nucleus (*17*), it is conceivable that the narcotic receptors responsible for the above effect are located presynaptically on dopamine nerve terminals.

The mechanism by which enkephalins stimulate dopamine synthesis is not known. It is possible that activation of opioid receptors at the dopamine nerve terminals results in a decreased release of dopamine which, in turn, increases dopamine synthesis because of removal of the normal inhibitory effect of dopamine on presynaptic receptors (*18*).

Consistent with this hypothesis is the report of Loh *et al.* (*19*) that morphine and β-endorphine inhibit potassium chloride–induced release of dopamine from striatal synaptosomes in vitro, an effect which is antagonized by naloxone. On the other hand, an inhibition of dopamine release might explain the cataleptic response to enkephalins (*5a*).

Our data indicate that the activation of narcotic receptors by the naturally occurring methionine-enkephalin may have a physiological role in controlling dopaminergic activity.

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21. This work was supported by Tecnofarmaci S.p.A., Pomezia-Roma. We thank A. Pletscher of Hoffman-La Roche, Basel, for the NDS 1015.

20 December 1977

Chemotactic Antibody

Abstract. *Antibody of the immunoglobulin G class to herpes simplex virus and antibody of the immunoglobulin M class to sheep red blood cells were coupled to the synthetic peptide formylmethionylleucylphenylalanine (fMet-Leu-Phe), which is chemotactic for both mononuclear and polymorphonuclear leukocytes. The resulting molecules were chemotactic and retained their antigen-binding activity. When antibodies coupled to fMet-Leu-Phe were incubated with antigen, the resulting immune complexes were also chemotactic. Chemotactic antibody may provide a potent means of enhancing the migration of inflammatory cells to specific sites.*

Inflammation is a common host response to a variety of disease processes. In recent years, a number of naturally occurring biological mediators that cause leukocytes to migrate have been identified (*1*). The resulting accumulation of leukocytes plays an important role in the defense against bacteria, viruses, and tumors. A deficiency in chemotactic factors generated locally may be the cause of inadequate numbers of leukocytes at the site of a lesion, resulting in failure to cope with certain infections or tumors.

A group of synthetic formylated (f) peptides of low molecular weight has been found to be chemotactic at concentrations of 10^{-6} to $10^{-11}M$ (*2*). One of the most active of these peptides is formylmethionylleucylphenylalanine (fMet-Leu-Phe) (*3*). If this small peptide could be coupled to antibody directed against a specific virus or tumor, the resulting

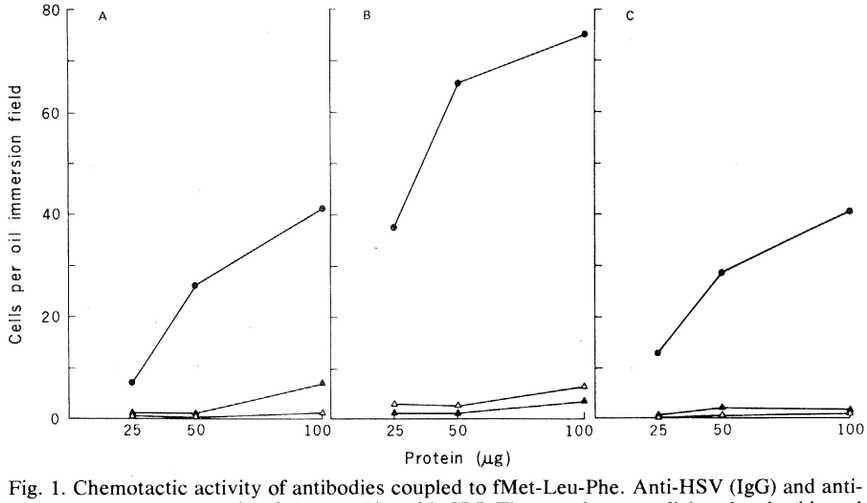

Fig. 1. Chemotactic activity of antibodies coupled to fMet-Leu-Phe. Anti-HSV (IgG) and anti-SRBC (IgM) were coupled to fMet-Leu-Phe with CDI. The samples were dialyzed and subjected to molecular sieve chromatography. The antibody-containing peaks were concentrated and assayed for chemotactic activity using guinea pig peritoneal macrophages. (A) (●—●) Anti-HSV coupled to fMet-Leu-Phe with CDI; (△—△) anti-HSV treated with CDI; (▲—▲) untreated anti-HSV. (B) (●—●) Anti-SRBC coupled to fMet-Leu-Phe with CDI; (△—△) anti-SRBC treated with CDI; (▲—▲) untreated anti-SRBC. (C) Chemotactic activity of immune complexes containing anti-SRBC and 0.083 mg of nitrogen from SRBC stroma. (●—●) Anti-SRBC coupled to fMet-Leu-Phe with CDI; (△—△) anti-SRBC treated with CDI; (▲—▲) untreated anti-SRBC. Each point represents the mean of duplicate samples.

0036-8075/78/0505-0554$00.50/0