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Contenuto

4 th SOVIET-ITALIAN SYMPOSIUM ON NEUROPSYCHOPHARMACOLOGY

Moscov, May 18–22, 1981

V. G. LONGO (*) e A. V. VALDMAN (**), Eds.

Contents

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SECTION III. – NEUROCHEMICAL AND NEUROPHARMACOLOGICAL ASPECTS OF PATHOLOGICAL CONDITIONS OF THE BRAIN

POSTER SESSION

Opening ceremony of the 4th Soviet–Italian Symposium on Neuropsychopharmacology: from left to right: Zakusov, Massotti, Anichkov, Mereu, Scapagnini, Valdman, Longo, Clementi, Pepeu, Annunziato, Gessa, Levi, Kharkevich, Moroni.

Protocol of the IV Soviet-Italian Symposium on the Pharmacology of the Central Nervous System

May 18–22, 1981, Moscow, USSR

In accordance with the Agreement on Cooperation between the USSR and Italy in the field of Health Care and Medical Science, the IV Soviet–Italian Symposium on the Pharmacology of the Central Nervous System was held in Moscow on May 18–22. It was organised by the Institute of Pharmacology of the Academy of Medical Sciences of the USSR with the assistance of the All-Union Scientific Society of Pharmacologists.

In the Symposium participated:

– from the Soviet side – 40 pharmacologists specializing in the pharmacology and physiology of the central nervous system headed by A. V. Valdman, Corresponding–Member of the Academy of Medical Sciences of the USSR.

– from the Italian side – 12 pharmacologists from Italy headed by Professor V. G. Longo.

Within the framework of the Symposium there were delivered 28 oral and 21 poster communications devoted to actual aspects of the study of the pharmacology of the central nervous system. They are: neurotransmitters and the mechanisms of action of psychotropic drugs, mediator aminoacids and other neurotransmitters, receptors of CNS and the effects of endo– and exogenous substances, neurochemical and neuropharmacological aspects of the pathological states of the brain.

During the Symposium there were discussed the results of the Soviet–Italian cooperation in the years 1979–1980. The most important of them are:

– the study, using the methods worked out by the Italian scientists and tried at the Institute of Pharmacology of the Academy of Medical Sciences of the USSR, of the pharmacokinetics of a number of benzodiazepine tranquilizers, such as: clobazam, diazepam and the first Soviet tranquilizer — phenazepam — in animals and man. This study makes it possible to use phenazepam efficiently in clinic;

– the new data on the role of the activity changes of the neurotransmitter systems of the brain in the processes of memory and learning disturbances and their possible pharmacological correction;

– the beginning of the joint research on the role of the specific proteins of the nervous system in the processes of memory and in the regulation of the immunologic reactions;

– 1979 in Italy / Cagliari / there was held the Soviet–Italian Symposium « Neurotransmitters and the Functions of the Brain » the proceedings of which were later published.

The Symposium promoted a comprehensive exchange of information on the above–mentioned pharmacological problems and confirmed high conceptual and methodical levels of the studies of both Parties in the field of the study of the mechanisms of action and the search of new neuropsychotropic drugs.

Taking in consideration the results of the cooperation and it's prospects, both Parties believe it useful to continue in 1981–1982 years the joint investigations in the following fields:

Theme 1: *Role of the Biogenic Amines in the Neurohumoral Regulation of the Brain Functions;*

Theme 2: *Research in the Field of Neuro– and Psychopharmacology;*

and the start of the cooperation on Theme 3: *Role of the Specific Proteins of the Nervous System in the Processes of Memory and in the Regulation of the Immunologic Reactions.*

This plan of joint studies includes joint research in the framework of the annual working–programs of the cooperating institutes, exchange of the specialists and scientific information, publication of joint articles.

Both Parties believe it useful to hold the V Soviet–Italian Symposium in 1983 in Italy.

Signed in Moscow on May 21, 1981 in two copies in Russian and in English, both being of equal validity.

Coordinator of the problem from the Soviet side Corresponding Member, Academy of Medical Sciences of the USSR

A. V. VALDMAN

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G. L. GESSA

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Protocol of the III Soviet-Italian Symposium on the Pharmacology of the Central Nervous System

June 4–7, 1979 Villasimius (Cagliari, Italy)

In accordance with the agreement of collaboration between the USSR and Italy in the field of medical science, the III Soviet–Italian Symposium on Neurotrasmitters and Brain Function was held in Villasimius (Cagliari, Italy), June 4–7, 1979. The Organizations responsible for the Symposium were the Italian Pharmacological Society, the Italian Ministry of Health, the Sardinian Health Office, the Mediterranean Foundation for Brain Research.

The following scientists participated in the works of the Symposium: from the Italian side, 52 pharmacologists, specialist in the field of pharmacology of the central nervous system; from the Soviet side, 5 pharmacologists from the Institutes and Universities of Moscow, Leningrad and Minsk, with prof. Zakusov as head of delegation.

The Symposium covered the following topics: Neurotransmitters and brain function; Peptides in neuroendocrine function; Gaba, serotonin, acetylcholine; Chronic treatments and neurotransmitters; Dopamine: receptors and metabolism, and included 12 main lectures and 44 communications.

Both sides have agreed to publish the proceedings of the Symposium in the official Journal of the Italian Pharmacological Society, Pharmacological Research Communication, possibly under the form of a special issue.

In order to implement the fellowship programme already mentioned in the previous protocol, a Committee has been established, formed by Prof. V. V. Zakusov and Prof. A. V. Valdman on the Soviet side, and by Prof. V. G. Longo and Prof. G. L. Gessa on the Italian side. Task of the Committee will be to secure ear–marked funds from the national agencies, make a survey of the institutes willing to receive and train fellows, provide adeguate dissemination of the announcements. The work of the Committee should be ratified at the next session of the Soviet–Italian working group for the collaboration in the field of health service and medical science.

Both sides have agreed that the next Soviet–Italian Symposium will be held in USSR in 1981.

Rome 8 June 1979

V. V. ZAKUSOV
A. V. VALDMAN
V. G. LONGO
G. L. GESSA

Opening Addresses

Dear participants and dear guests,

today we are opening the IV Symposium on Neuropsychopharmacology organised within the framework of the Soviet–Italian cooperation in field of Pharmacology. May I remind here briefly that according to our official agreements there are several institutes engaged in this joint venture. From the Soviet side: the Institute of Pharmacology of the Academy of Medical Sciences of the USSR, the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR and the Laboratory of Biochemistry of Neurohormones of the Minsk Medical College; from the Italian side: the Istituto Superiore di Sanità (Rome), the Institute of Pharmacological Research « Mario Negri » (Milan), the Institute of Pharmacology of Milan University, the Institute of Pharmacology of Cagliari University.

Research was carried out in several directions:

- Role of the biogenic amines in the regulation of organism functions;
- Neuro– and psychopharmacology;
- Role of specific proteins of the nervous system in the processes of memory and in the regulation of the immunologic reactions.

During the organization of these years one of the most useful forms of cooperation was Symposia, where the Soviet and Italian scientists presented the results of their research, exchanged ideas, worked out joint investigations.

The first Symposium was held in Modena in 1976. The second was held in Moscow in 1977, the proceedings of this Symposium were published in a special volume of the Annali of the Istituto Superiore di Sanità. The third one was held in Cagliari in 1979 and also in this case the proceedings were published in a special issue of the official Journal of the Italian Pharmacological Society.

In addition to symposia, a number of visits have been arranged, so that scientists from the Institute of Pharmacology and from the Institute of Experimental Medicine of the Academy of Medical Sciences as well as from the Minsk Medical College had the opportunity to learn some new methods in the neurochemical and neuropharmacological field.

Opening the Symposium, first of all let me thank the Italian coordinator Prof. Longo and the other Italian pharmacologists who have accepted our invitations and are going to present their work at this symposium, which will cover the following topics:

- neurotransmitters and the mechanisms of action of psychotropic drugs;
- receptors of the CNS effects of endo– and exogenous substances;
- neurochemical and neuropharmacological aspects of the pathological states of the brain;
- oligopeptides and other neurotransmitters.

Let me wish the participants fruitful scientific discussion and give the floor to Prof. Longo.

A. V. VALDMAN

It is a great pleasure for me to be present with my colleagues at this fourth Italo Soviet Symposium on Neuropsychopharmacology. I would like to call your attention that on this year falls the 10th anniversary of the scientific agreement signed in Rome by the representatives of the Italian and Soviet government, therefore we are adeguately celebrating this important event.

Sufficient experience has already gained to justify the conclusion that this type of exchange is an effective way of improving understanding among scientists, of changing attitudes and of overcoming prejudices. I would like to add that the quality and quantity of the scientific contributions has grown steadily in these years, bringing them to an excellent level which we must strive to maintain in the future. On the other hand, I regret than the program of fellowships which was included in the protocols signed in Moscow in 1977 and for which in Cagliari was proposed a special committee, did not progress satisfactorily. Since I am convinced that such an implementation of our collaborative program is an important step forward, may I call on the help of everybody present in order to start such an exchange.

V. G. LONGO

with morphine. Furthermore, dermorphin shows a long duration of action due to its resistance to enzymatic hydrolysis for the presence of a *D*-Ala² residue in its molecule [2].

In the present investigation the effects of intracerebroventricular administration of dermorphin and of two dermorphin derivatives on the electrical cerebral activity (EEG) and behavior of rats and rabbits were studied. The action of the specific opiate antagonist, naloxone, on dermorphin-induced changes was also determined. In a separate series of experiments mice were used to study the influence of dermorphin, compared to that of morphine, on behavior and in particular on the Straub tail reaction. The two dermorphin derivatives were:

FCE 21127 = H-TYR-ALA-PHE-GLY-TYR--PRO-SER-NH-Me (Methyl dermorphin)

FCE 21547 = HCl. H-TYR-ALA-PHE-GLY-TYR (BZL)-PRO-SER (BZL)-NH_2 (dibenzyl dermorphin).

Methods.

Twelve adult male rabbits weighing 2.8 to 3.2 kg were used. Electrodes for EEG recording were chronically implanted under pentobarbital anesthesia epidurally over the cortex and in the hippocampus. Electrodes were attached to the skull with dental cement. For drug injection, a canula was permanently inserted into the lateral cerebral ventricle according to a technique described in a previous work [3].

A total of 20 Wistar male rats weighing 250–350 g were used. Four electrodes were chronically implanted under pentobarbital over the cortex. A permanent canula was inserted into the right cerebral ventricle using the stereotaxic coordinates from the Atlas of de Groot [4]. The location of the canula was controlled *post mortem* by examination of the ventricles for the presence of China ink injected just before sacrifice.

After recovery from surgery (7–10 days), the animals were put in a shielded cage (1 m × 1 m × 0.65 m) and connected to the apparatus by means of long wires, thereby permitting free movements. For recording an 8-channel EEG Galileo apparatus model E 10 B was used. The volume of intraventricular injection did not exceed 20 µl for rats and 40 µl for rabbits; the same volume of saline solution was injected during the control recordings. The animals were treated with the compound at 8–10 days intervals in order to limit the development of tolerance. Each animal was treated 2–4 times. During the EEG experiments, concomitant observations of behavior were carried out, covering a subjective evaluation of the animals reaction to noxious stimuli. Animals were also observed for the occurrence of rigidity, catatonia and reactions to auditory and tactile stimulation. Rigidity was evaluated by subjective assessement during handling; for the evaluation of catatonia the rat's forelimbs were placed on a bar 7 cm high, and the time of immobility was measured; rabbit's hind limbs were placed on a wooden box 15 cm high. Animals were considered catatonic when remained in the imposed posture for at least 30 seconds. Both rabbits and rats were considered as having lost the righting reflex if they kept the supine position for at least 30 seconds. Analgesia was assessed by pinching with an alligator clamp the ear in the rabbit and the tail in the rat.

In a separate series of experiments, 6 adult male rabbits were used for the topical application of dermorphin and morphine. Under ether anesthesia a small area of the sensorimotor cortex of one side was exposed. The effect of anesthesia was allowed to wear off; the animal was then placed in a restraining box and the electrical activity was registered both from the exposed area, using a silver wire lightly applied to the cortex, and from other zones using screw electrodes implanted epidurally over the cortex. Filter paper discs soaked with solutions of dermorphin (0.5, 1.0 and 2.0 %) and morphine (0.5 and 1.0 %) were used. To insure the continuous effect of the drug over the cortex the discs were left *in situ* during the entire experiment and replaced when necessary. A disc of saline solution was applied as control prior to drug application.

A total of 450 male Swiss mice, weighing 20–25 g, were used. The animals received intracerebral injection using the method of Haley and Mc Cormick [5]. Drugs were injected to groups of 5 animals. The volume of drug administration was 10 µl. Control animals received the same volume of saline. Immediately after treatment the mice were put in a wooden observation cage (32 × 32 cm) and, during the first hour after injection, were evaluated every ten minutes for the presence of the running fits and of the Straub tail reaction (STR). The elevation of the tail at angles of 90° or greater has been used to define the tail erection response. The method of analysis utilized was the Fisher exact test.

Natural and synthetic dermorphin and dibenzyl dermorphin were prepared in a solution containing 50–60 % of ethanol, which was evaporated under vacuum on a rotavapor. The residue was dissolved in saline; only freshly prepared solutions were used. Methyl dermorphin was dissolved in saline. Naloxone hydrochloride was diluted in distilled water and administered intravenously in the rabbit and intraperitoneally in the rat and in the mouse. Morphine hydrochloride was dissolved in saline and administered in mice both intracerebrally and intraperitoneally. Scopolamine hydrochloride was dissolved in distilled water. The doses of morphine, naloxone, and scopolamine referred to in the text are given in terms of the weight of the base. Scopolamine and naloxone were administered intraperitoneally to mice 15 minutes before dermorphin or morphine treatment.

Results.

Rabbits

Rabbits were injected intracerebroventricularly with doses of dermorphin ranging from 0.05 to 30 µg. No appreciable changes were observed on the EEG record and behavior after administration of 0.05 µg. Injections of higher doses (0.1, 0.2, 0.5 and 1.0 µg) induced a period of desynchronization (15–25 min) during which the animals showed mydriasis, exophtalmus, absence of spontaneous movements and loss

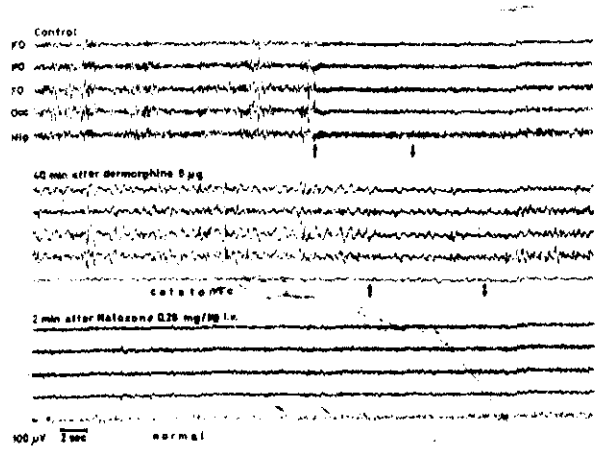

FIG. 2. – Effects of dermorphin administered into the lateral cerebral ventricle of the rabbit bearing chronically implanted electrodes and cannula. Antagonistic action of naloxone. *Upper record*: control, the acustic stimulation (arrows) induces the activation of the tracing. *Middle record*: forty minutes after i.c.v. administration of dermorphin (5 µg) EEG synchronization with slow components is present, the theta waves are disrupted and the arousal reaction is blocked. *Lower record*: naloxone (0.25 mg/kg, i.v.) induces a complete EEG and behavioral recovery. *Leads*: FP: anterior-posterior sensorimotor cortex; PO: posterior sensorimotor-optic cortex; Occ: optic cortex; Hip: dorsal hippocampus.

of response to noxious stimuli. This phase was followed by a synchronous EEG pattern associated with muscular rigidity. Increasing the dose of dermorphin from 0.1 to 1.0 µg resulted in an increase in the duration of EEG synchronization to a maximum of 60–90 minutes at the higher dose. The dose of 1.0 µg induced a slight lowering in voltage and frequency of the « theta » waves in the hippocampal lead. The EEG arousal response was sometimes attenuated. Another phenomenon which was always observed, often before the appearance of the synchronous pattern, was the « paradoxical EEG response » previously described by Carruyo et al. [6] in rats treated with opiates: upon pinching of the ear slow waves appeared in the record, disappearing at the end of the stimulus. Doses of 2.5 and 5.0 µg (Fig. 2) induced within a few minutes the appearance of a synchronous EEG pattern with high voltage slow waves in the cortical leads and the complete disruption of « theta » waves in the hippocampal lead. The EEG arousal response was often blocked. Behaviorally, the animals showed a marked muscular rigidity, catatonia and loss of the righting reflex. These effects lasted about 3–4 hours. Increasing doses up to 10 µg induced within 10–20 minutes the appearance of slow waves; during this phase the arousal response was not elicitable. The « theta » waves in the hippocampal lead were completely disrupted. The effects on behavior, already described, were much more intense and long lasting (about 5–6 hours). Higher doses, 20 and 30 µg, caused death of some animals. Death occurred 1–2 days after treatment and was preceeded by flaccid paralysis of the hind limbs. These doses induced also the appearance of rare spike-wave complexes in the record. The topical application of dermorphin over the sensorimotor cortex at concentrations of 0.5, 1.0 and 2.0 % caused a highly disorganized activity consisting exclusively of slow waves; motor or EEG convulsive manifestations were never observed. Morphine, (0.5–1 %) induced the appearance of spikes after a variable delay (10–20 min). Clonic movements of the muzzle were seen synchronous with the spikes. Spikes were, at first, localized in the exposed cortex and then spread to the other leads, leading in some instances ot generalized seizures.

Rats

Rats were treated with doses of dermorphin ranging from 0.005 to 2.5 µg. Immediately after injection or within a few minutes, all doses gave rise to grand-mal EEG seizures more or less sustained according to the dose, associated with motor manifestations (Fig. 3); these consisted, with the low doses, of twitches and slight tremors; tonico-clonic convulsions appeared with the higher doses. The convulsive patterns were followed by high voltage slow waves, interrupted by periods of low voltage desynchronous EEG with scattered spikes. Blockade of the activation to external stimuli and the « paradoxical EEG response » were always present. During the postconvulsive phase induced by low doses of dermorphin the animals showed hyperreactivity; after high doses they showed absence of spontaneous movements, exophtalmus, mydriasis, rigidity and catatonia. The EEG and behavioral modifications persisted for 30 minutes at the lowest dose (0.005 µg), and several hours (6–8) at the highest doses. The dose of 2.5 µg induced marked hypothermia and respiratory depression and the animals died within 24 hours from treatment. Methyl dermorphin showed pharmacological properties very similar to that of dermorphin, namely analgesic and convulsant properties at the doses of 0.3 µg.

On the other hand, dibenzyl dermorphin was devoid of analgesic property and induced convulsions only at high doses (20–30 µg).

Naloxone, administered intravenously in the rabbit at doses of 0.25, 0.5 and 1.0 mg/kg, was able to antagonize all the EEG and behavioral effects induced by dermorphin. The antagonistic effect of naloxone

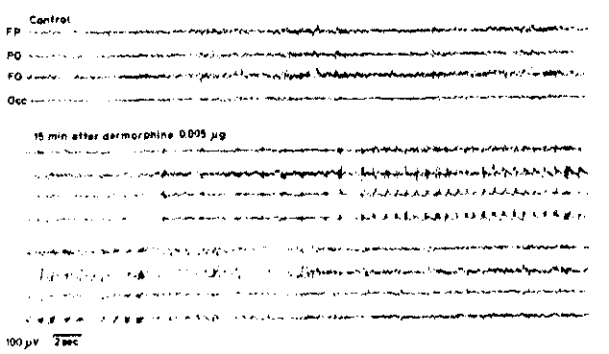

FIG. 3. – Effects of dermorphin administered in the lateral cerebral ventricle of the rat bearing chronically implanted electrodes and cannula. *Upper record*: control EEG. *Middle and lower records*: fifteen minutes after i.c.v. administration of dermorphin (0.005 µg) bursts of spikes appear in the tracing. Twitchings and tremors accompany the electrical manifestations. The two records are continuous and have been divided only for convenience. *Leads*: FP: anterior-posterior sensorimotor cortex; PO: posterior sensorimotor-optic cortex; FO: anterior sensorimotor-optic cortex, Occ: optic cortex.

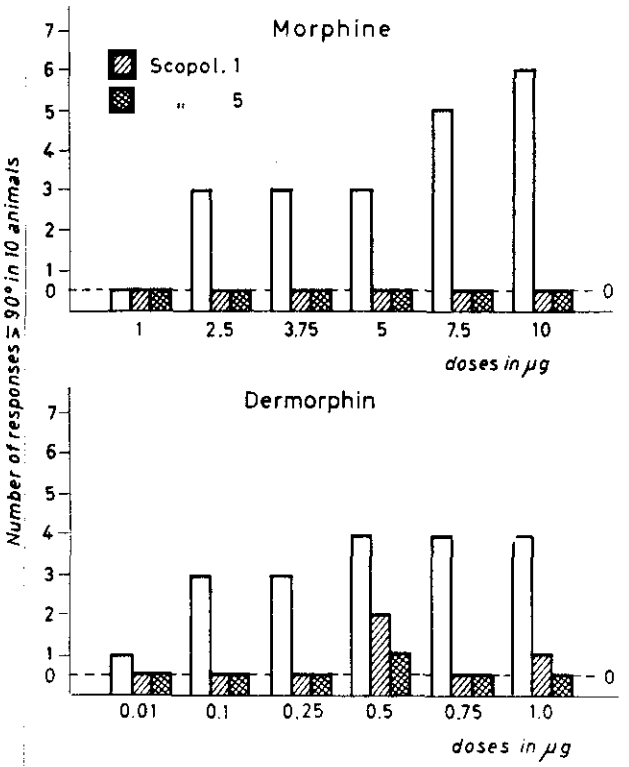

FIG. 4. – Straub tail reaction after intracerebral administration of morphine and dermorphin. Antagonistic effects of scopolamine. Ordinates: number of responses $\geq 90^{o}$ in 10 animals. Abscissa: doses in μg. Values are the average of two experiments (5 animals per experiment per dose). Scopolamine (1–5 mg/kg i.p.) antagonizes morphine and dermorphin induced tail erection.

(1 mg/kg) was transient (10–20 min) when high doses of dermorphin (20–30 μg) had been previously injected. In the rat, repeated injections of 0.5 or 1 mg/kg were necessary to reverse permanently or transiently the effects of dermorphin.

Mice

Dermorphin was administered intracerebrally to mice at doses of 0.01, 0.1, 0.25, 0.5, 0.75 and 1.0 μg. All the doses induced an increase in the number of mice with a tail elevation $\leq 90^{o}$ compared with the control mice, but statistically significant differences were found only with the higher doses (Fig. 4). Doses from 0.01 to 1.0 μg produced in 100 % of animals restleness and increased locomotor activity (running fit). At doses higher than 1.0 μg mice were cataleptic, and did not exhibited the STR or the running fit. Morphine was injected intracerebrally in doses of 1.0, 2.5, 3.75, 5.0, 7.5 and 10 μg. Doses of 5.0, 7.5 and 10 μg induced tail elevation of 90^{o} or greater respectively in 4,5 and 6 mice out of 10 with a median latency of 20 min. Morphine induced the running fits at all the doses. Doses higher than 10 μg produced only catalepsy. The effects of scopolamine, in doses of 1 and 5 mg/kg i.p., on morphine and dermorphin induced tail elevation are shown in Fig. 4. Pretreatment with scopolamine strongly reduced the number of mice with a STR $\leq 90^{o}$; this effect was more evident in the animals treated with morphine. Pretreatment with naloxone (1 mg/kg, i.p.) completely antagonized the effects of all doses of morphine and dermorphin on the STR.

DISCUSSION.

Comparison of the present results with those of a previous study carried out in this laboratory [3] reveals that dermorphin induces in the rabbit EEG and behavioral modifications which closely resemble those occurring after treatment with some structural analogues of met–enkephalin (FK–33824 and *D*–Ala–enkephalinamide). On the other hand, differences are found comparing the EEG patterns observed after dermorphin and that occurring after morphine. Morphine, administered i.c.v. (25 μg) gives rise to behavioral alerting and EEG desynchronization followed by tipical « grand mal » EEG seizures, while dermorphin, as well as the synthetic enkephalins, is devoid of convulsant activity. These findings are further supported by the data obtained in acute experiments following topical application of dermorphin over the sensorimotor cortex of the rabbit. Up to concentration of 2 % the peptide is devoid of convulsant activity, while morphine applied topically at concentrations of 0.5–1.0 % induces the appearance of spikes. Intracerebroventricular administration of dermorphin, enkephalins and morphine in the rat gives rise to epileptic phenomena immediately after treatment. These findings are in agreement with those reported by Urca et al. [7] after i.c.v. administration of morphine and met–enkephalin and by Tortella et al. [8] after morphine and *D*–Ala–enkephalinamide. Dermorphin is respectively 2500 and 10000 times more potent than morphine and the natural enkephalins in inducing epileptic fits in the rat [3]. The synthetic peptide FK 33824 is slightly more potent than dermorphin, infact, the EEG and behavioral alterations induced by FK 33824 are more intense and long lasting than those occurring after the same doses of dermorphin.

Present results indicate that dermorphin causes EEG and behavioral epileptic phenomena in the rat but is devoid of convulsant activity in the rabbit. The reason of the different action of dermorphin in the two animal species remains to be elucidated. However, recent findings obtained in pharmacological assays [9] have indicated that opioid peptides interact with at least two receptors: the μ receptors, to which morphine binds preferentially, and the δ receptors to which enkephalins have a preferential affinity. Furthermore, Kosterlitz [10] has reported that naloxone has a low effectiveness against the action of enkephalins (δ receptors) and, as a specific opiate antagonist, interacts mainly with μ receptor. Various pharmacological effects of enkephalin derivatives suggest that μ receptors mediate the analgesic action of opiates while δ receptors may be responsible for epileptic and behavioral effects [11, 12]. Hence, it could be hypothesized that the different patterns observed in rats and rabbits are probably due to the binding of dermorphin to different opiate receptors in the central nervous system of the two animal species. In the rat, dermorphin could bind equally to μ and δ receptors (both analgesic and epileptic activity) while in rabbits it could act preferentially on μ receptors (strong analgesic activity and

absence of epileptic phenomena). This hypothesis is supported by the finding that naloxone often fails to antagonize the effects of dermorphin in rats at doses which are always effective in the rabbit.

Results of the study carried out in mice indicate that dermorphin elicits a Straub–tail reaction that is elicited by morphine and other narcotic drugs [13, 14].

Increasing the doses both of dermorphin and morphine does not cause an increase in responders. This is probably due to the fact that, when injected intracerebrally, the catatonic effect of the drugs overrides all other motor responses. The inhibition obtained with scopolamine may be correlated with its action on the opiate transmitter system. On the other hand this result is not in agreement with results obtained by Lee et al. [15], who found that tail erection induced by morphine (100 mg/kg s.c.) is not affected by pretreatment with large doses of cholinergic blocking agents such as atropine (20 mg/kg, i.p.), on the contrary is inhibited by neuroleptic drugs which are dopamine receptor blocking agents.

In conclusion, dermorphin can be classified as one of the most active peptide possessing peripheral and central opiate-like activities. The only peptide comparable to dermorphin for the intensity of its action is dynorphin 1–13 [16] and FK 33824. Dynorphin is rapidly degraded *in vivo* by the proteolytic enzimes and its central effects are of very short duration, while dermorphin has a very prolonged action and, at the highest doses tested (300 times higher than the analgesic dose) it seems to induce irreversible damages to the central nervous system.

Acknowledgments.

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The effect of motropine and of some neuropeptides on opiate receptors

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Summary. – *In this paper are reported the results obtained studying the influence of motropine, $ACTH_{4-7}$, β-MSH_{6-8}, a MIF analog and bradykinin$_{7-9}$ on the analgesia due to morphine.*

Motropine (10 mg/kg i.v.) noticeably reduced the morphine and enkephalin-induced analgesia, showing an effect similar to that of thyroliberin (TRH). $ACTH_{4-7}$, MSH_{6-8}, the MIF analog and bradykinin$_{7-9}$ also antagonized the analgesic effect of morphine but to a lesser degree.

Motropine and $ACTH_{4-7}$ reduced the duration of hexobarbital sleep. The other neuropeptides were less active in this respect. Bradykinin$_{7-9}$ had no effect.

The data suggest that motropine and some hypothalamic neuropeptides have similar features with respect to their action on opiate pain receptors.

Riassunto. – *Sono stati studiati gli effetti centrali della motropina, $ACTH_{4-7}$, β-MSH_{6-8}, bradikinina$_{7-9}$ e di un analogo del MIF, ed in particolare il loro antagonismo nei riguardi della morfina.*

La motropina attenuava l'azione analgesica della morfina dimostrando notevoli analogie col TRF; gli altri prodotti possedevano lo stesso effetto ma meno marcato. La motropina e l'$ACTH_{4-7}$ riducono il sonno da barbiturici. Questi dati suggeriscono che la motropina ha molte proprietà in comune con i neuropeptidi ipotalamici.

Introduction.

Motropine (tropyl ester β-N-morpholyl propionic acid dihydrochloride) and other tropine derivatives substituted in C_3 by acyloxyl radicals are endowed with nervous activity stimulant activity and antagonize the analgesic effect of morphine, of other narcotic analgetics as well as of the opioid tetrapeptide Tyr-D-Ala-Gly-Phe-NH_2 [1, 2]. For istance, motropine (10 mg/kg i.p.) causes in rats a marked decrease of the threshold of vocalization to the electrical stimulation of the tail raised by previous treatment with morphine (2.5 mg/kg i.v.) or with the tetrapeptide (10 mg/kg i.v.). In rabbits motropine (4 mg/kg, i.v.) restores the impulse summation reduced or suppressed by morphine (0.5–1 mg/kg, i.v.); when motropine is administered prior to morphine, no reduction of the impulse summation occurs. The same antagonism is observed with respect to the effect of other opiates (azidomorphine, trimeperidine) and of the tetrapeptide opioid. In this test motropine is only slightly less potent than nalorphine and naloxone, while in the rat tail stimulation test it is much less potent.

Thyroliberine (pGlu-His-Pro-NH_2) and some other neuropeptides, *e.g.*, adrenocorticotropic hormone (ACTH), β-melanocyte stimulating hormone (β-MSH), melanostatin (MIF), bradykinin, show a certain antagonism to the effects of opiates and opioid peptides [3, 4]. In this paper are reported the results obtained studying the effects of motropine, $ACTH_{4-7}$, β-MSH_{6-8}, MIF analog, bradykinin$_{7-9}$ on the analgesic effect of morphine and enkephalin analogs.

Methods.

The stimulating activity of morphine and of the neuropeptides was estimated by their effects on spontaneous and amphetamine-potentiated locomotor activity and on the duration of hexobarbital sleep in mice. Locomotor activity was recorded for 30 min using Opto Varimex. Motropine and the neuropeptides were injected intravenously (in the tail vein); amphetamine (5 mg/kg) was administered subcutaneously; hexobarbital (50 mg/kg) was administered intraperitoneally.

In rats, vocalization during electrical stimulation of the tail was used a criterion for analgesia. Motropine and the neuropeptides were administered 15 min after the opiates. Morphine, the tetrapeptide opioid and its nitroanalog were injected i.v. in doses of 2.5; 10; 2 mg/kg, respectively.

The effect of motropine and the neuropeptides on the respiratory depression induced by morphine was studied in rabbits. The amplitude and rate of respiration movements were recorded using a Volucapt transducer and registered on paper. The compounds under study were administered i.v.

Results and Discussion.

Motropine, thyroliberine, bradykinin$_{7-9}$ and the MIF analog did not increase spontaneous activity in mice but produced a marked potentiation of the locomotor stimulation due to amphetamine. $ACTH_{4-7}$ and β-MSH_{6-8} (5 mg/kg i.v.) had a weaker effect (Fig. 1).

Motropine and $ACTH_{4-7}$ reduced the duration of hexobarbital sleep. The other neuropeptides were less active in this respect. $Bradykinin_{7-9}$ had no effect (Fig. 2).

Motropine (10 mg/kg i.v.) noticeably reduced the analgesic effect of morphine. Thyroliberine (TRH) acted in a similar manner and at the same dose produced a marked reduction of analgesia (Fig. 3). $ACTH_{4-7}$, MSH_{6-8}, the MIF analog and $bradykinin_{7-9}$ also antagonized the analgesic effect of morphine but to a lesser degree.

In rabbits, morphine and the tetrapeptide opioid (1–2 mg/kg i.v.) decreased the amplitude and rate of respiration by about 50 %. Thyroliberine (4–5 mg/kg i.v.) eliminated these effects restoring the volume, amplitude and rate of respiration almost to the initial level (Fig. 4). $ACTH_{4-7}$, β-MSH_{6-8}, the MIF analog, $bradykinin_{7-9}$, motropine had no effect. Thus, thyroliberine eliminated the analgesic effect of morphine and their depressing effect on respiration.

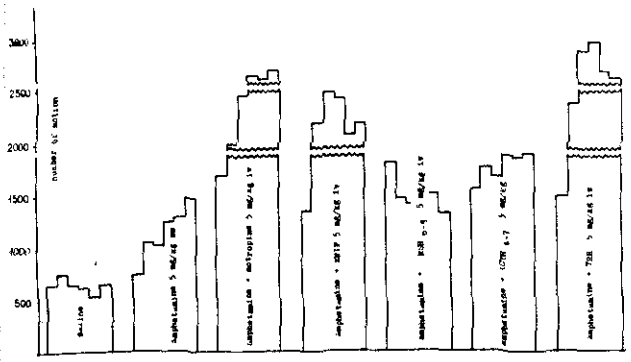
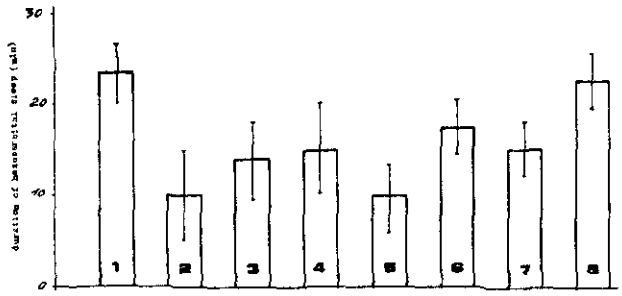


FIG. 1. – Potentiation of amphetamine-enhanced locomotor activity in mice by neuropeptides.



1 hexobarbital (H) 59 mg/kg i.p.
2 H + motropine 10 mg/kg i.v.
3 H + TRH 5 mg/kg i.v.
4 H + naloxone 5 mg/kg i.v.
5 H + ACTH 4–7 10 mg/kg i.v.
6 H + β-MSH 6–8 10 mg/kg i.v.
7 H + xMIF 10 mg/kg i.v.
8 H + bradykinin 7–9 10 mg/k i.v.

FIG. 2. – Influence of neuropeptides on the duration of hexobarbital sleep.

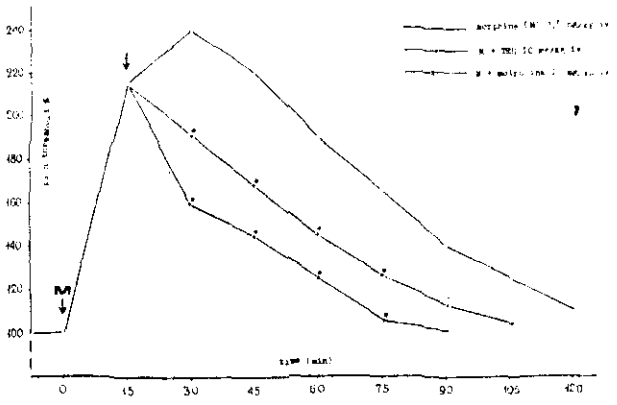
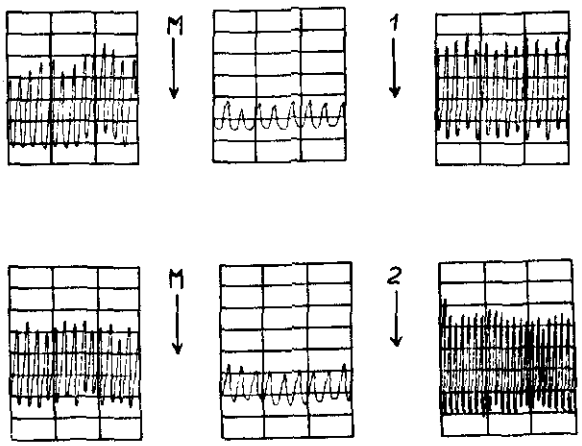


FIG. 3. – Effects of motropine and TRH on the analgesic action of morphine in rats.



M – morphine 2 mg/kg i.v.

1 – TRH 5 mg/kg i.v. 2 – nalorphine 3 mg/kg i.v.

FIG. 4. – Effects of TRH and nalorphine on the amplitude and rate of respiration of the rabbit.

All neuropeptides except $bradykinin_{7-9}$ and morphine showed a marked antagonism with hexobarbital.

Our data suggest that motropine and some neuropeptides have similar features with respect to their action on opiate pain receptors. This is confirmed by the results of the study of morphine effect on electropuncture analgesia in rabbits. Research in progress [5] indicated that motropine as well as naloxone completely eliminates the analgesic effect in the tail-flick test and also the changes of evoked potentials in the somatosensory area of the cerebral cortex caused in rats by electroacupuncture. This suggests that motrophine antagonizes the endogenous opiate neuropeptides of enkephalin type formed during electroacupuncture.

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Psychotropic activity of the natural peptide tuftsin

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Summary. – *The present study indicates that tuftsin, a tetrapeptide with a phagocytosis-stimulating effect, produces central stimulation, allegedly mediated by the CA brain system. Tuftsin's activating effect on emotional reactivity and behavior is partly similar to the effect of some other small peptides (TRH, MIF) having common or equipotent structural fragments. According to unpublished data, some tuftsin analogs also have a central stimulating effect. This is promising view of further research for central stimulants among « small peptides ».*

Riassunto. – *La tuftsina (Treo-Lis-Pro-Arg) é un tetrapeptide isolato dell'immunoglobulina umana IgG che possiede proprietà immunostimolanti. Nel presente lavoro sono stati studiati gli effetti centrali della tuftsina nei topi e nei ratti. Il farmaco ha dimostrato proprietà stimolanti non dissimili da quelle del MIF e di altri oligopeptidi.*

Introduction.

Tuftsin (Thr–Lys–Pro–Arg) is a tetrapeptide isolated from a heavy chain of human immunoglobulin IgG [1]. *In vivo* and *in vitro* tuftsin increases phagocytosis, migration of polymorphonuclear human leucocytes and triggers immunological functions. Radioimmunoassay reveals the presence of tuftsin in blood of healthy subjects (255 μg/l). The structure of tufsin conforms to the model of functional organization of low-molecular peptide ligands [2], whose common fragments contain identical or equi-functional amino acids. These peptides have various biological activities, participate in the ligand-receptor interaction and probably in the intracellular information transfer.

It has been previously shown that some small peptides, such as the C-terminal fragments of oxytocin$_{7-9}$ (also called melanostatin or MIF) and gastrin$_{15-17}$ activate emotional behavior [3] and affect biogenic amine metabolism in the brain [4]. In the present paper we report some data on the psychotropic effects of tuftsin.

Results and Discussion.

The effect of tuftsin was evaluated according to the method of Porsolt et al. [5], which induces behavioral depression through a non-avoidable stress situation (behavioral despair). Male mice (CBWA) were used. Tuftsin (20–200 μg/kg i.p.) showed a dose-dependent activating effect, decreasing the total duration of behavioral immobilization and prolonging the period of active behavior. Doses of 500 μg/kg induced sedative effects (Tab. 1).

Table 1. – *Effect of tuftsin on the total duration of immobility during a 6 min test.*

DRUG	Dose μg/kg	Duration of immobility (sec) Mean (S.E.M.)	% Control
Saline	—	174,0 (14,1)	100,0
Tuftsin	20	77,2 (14,5)	—66,0
	50	81,7 (19,4)	—53,0
	250	113,3 (24,7)	—35,0
	500	179,2 (13,3)	+ 2,9
Saline	—	130,0 (16,8)	100,0
Tuftsin	250	91,0 (12,9)	—30,0
Reserpine (4h before)..	2.500	255,0 (25,6)	+96,0
Reserpine + ...	2.500	93,0 (22,1)	—29,0
Tuftsin	250		

Male mice (CBWA) n = 6 per group.

Animal behavior in the Porsolt test is activated by substances with antidepressant activity and by psychostimulants [5, 6]. It was suggested that the activation of the catecholaminergic (CA) system is the neurochemical basis for this effect.

Since the activating effect of tuftsin could be related to the same CA mechanisms, tyrosine hydroxylase (TH) activity was estimated in rats pretreated with 500 μg/kg of tuftsin (i.p.). Solubilized brain tissue homogenates of hypothalamic and striatal areas were prepared. The activity of the enzyme was measured by direct spectrophotometric method [7]. Ten and 20 minutes after tuftsin administration TH activity in the hypothalamus and in the striatum increased (Tab. 2).

Different degrees of the activation of hypothalamic CA processes (mainly NE systems) and striatal ones (mainly DA systems) were found. Tuftsin-induced changes in the *in vivo* activity of TH reflect a short-

Table 2. – *Effect of acute tuftsin treatment on tyrosine hydroxylase activity in rat brain areas.*

	Reaction rate, nmoles/mg protein/min (M ± m)	
	Hypothalamus	Striatum
Control	16,0± 1,0	33,3± 2,8
Tuftsin, 10 min	25,0± 6,5	87,1± 6,2
Tuftsin, 20 min	44,3±14,4	55,7±11,1

The reaction rate was measured in solubilized brain tissue homogenates. Direct spectrophotometric method. Buffer 0,05 M tris–maleate, pH 6,1; 0,11 mM tyrosine; 0,17 mM 6,7–dimethyl-5,6,7,8–tetrahydropterine, 30 °C.

term regulation of kinetic properties of the rate–limiting enzyme of CA biosynthesis. It can be assumed that these adaptive changes result from an increased CA turnover, thus indicating a central action of tuftsin.

The contents of rat brain biogenic amines and their metabolites were estimated 10 and 20 min after tuftsin microinjection (10 μg) in the brain ventricle. Fluorimetric technique was used (Hitachi MPH–2A). Serotonin and 5–hydroxy–indolacetic acid were measured according to Curzon and Green [8]; dopamine, homovanillic acid and norepinephrine were measured according to Schellenberger and Gordon [9]. No significant alterations of brain amines level were found, although there was a tendency to HVA increase.

The circling behavior model [10] and drug–induced behavior were used to determine tuftsin's action on DA systems. Male Wistar rats were pretreated with 6–hydroxydopamine (16 μg) microinjection to the right caudate. Unilateral lesions of the DA terminals developed. Tuftsin (200 μg/kg i.p.) increased rotation toward the side of the lesion and increased the emotional reactivity (handling test, boxing in pair). Tuftsin diminished amphetamine–induced ipsilateral circling, but intensified stereotyped behavior (Tab. 3). Apomorphin–induced contralateral circling was not influenced. Tuftsin had a poor effect on behavioral depression induced by haloperidol, but enhanced circling and aggressiveness depressed by pretreatment with diethylditiocarbamate (a dopamine–beta–hydroxylase inhibitor). All this indicates a specific pattern of DA activation in the meso–limbic and nigro–striatal systems.

Tuftsin (500 μg/kg) increased emotional reactivity, estimated by measuring the threshold of pain reaction (vocalization) in rats upon electrostimulation of the paws. It also decreased the threshold of aggressiveness and fighting elicited by placing pairs of rats in cages with electrified floor. Tuftsin did not improve the learning of conditioned passive avoidance reaction to single negative stimulus with subsequent testing after 24 h when administered 10 min before testing (day 1). On the contrary, the number of animals with learned passive avoidance reaction diminished and the latency of the first passing to the dark chamber decreased. Tuftsin showed no marked myorelaxant effect.

Tuftsin (500 μg/kg) induced an activating effect with increased motivations and locomotor activity in model behavioral depression in cats. Two models were used: behavioral depression induced by reserpine (0.1 mg/kg s.c.) and behavioral inhibition which developed during and after stimulation of the medial septal area (20 Hz, 1.5 ms, 3 min); for details of this technique see [11].

The effect of tuftsin on animal behavior in a stress situation was estimated on the basis of the initial behavioral reactivity. Intact rats and rats with destroyed CA terminals (6–OHDA pretreatment in the neonatal

Table 3. – *Effect of tuftsin on circling and drug induced behavior.*

DRUGS	Doses (mg/kg)	Time after pretreatment (min)	Circling behavior (counts/5 min ± SEM)		Stereotyped behavior	Aggressive behavior
			Ipsilateral	Contralateral		
Saline	—	—	14,6±5,1	no	no	no
Tuftsin	0,2	—	23,7±4,5*	no	no	0,2±0,04*
Amphetamine	1,0	—	44,2±8,6	no	4,1±1,2	1,5±0,6
Amphetamine	1,0	—	27,4±5,3*	no	7,7±2,8*	0,4±0,06*
+ Tuftsin	0,2	15	—	—	—	—
Apomorphine	0,5	—	no	48,4±0,1	7,1±0,8	4,0±1,6
Apomorphine	0,5	20	no	53,3±9,4	6,0±3,1	2,2—0,9*
+ Tuftsin	0,2	—	—	—	—	—
Haloperidol	1,0	—	no	no	no	no
Haloperidol	1,0	—	3,0±0,9*	no	no	1,1±0,3*
+ Tuftsin	0,2	45	—	—	—	—
Diethylditio–carbamate (DTC)	35,0	—	8,3±2,4	no	no	no
DTC	35,0	25	27,1±5,4*	no	no	2,3±0,7*
+ Tuftsin	0,2	—	—	—	—	—

Rats (Wistar). Pretreatment (24h) – microinjection of 6–OHDA 16 μg in 4 μl saline to the right caudate.
(*) $p < 0,05$.

Table 4. – *Effects of tuftsin on avoidance behavior.*

GROUP OF ANIMALS	Type of reactivity	Unsuccessful attempts of avoidance (number)	Level of emotional manifestations (points)	Latency of escape (sec)
	1. *Saline*			
Intact	non-emotional	no	no	11,7±3,7
	emotional	16,4±3,8	7,2±3,0	39,8±5,0
6-OHDA Pretreatment	non-emotional	7,1±2,2	4,5±1,6	34,3±4,6
	emotional	26,4±5,3	14,1±2,7	68,2±6,1
	2. *Tuftsin 200 μg/kg*			
Intact	non-emotional	no	no	5,1±1,7
	emotional	4,8±1,6**	5,0±1,7	23,2±4,8
6-OHDA Pretreatment	non-emotional	no	2,9±0,8	19,2±3,5*
	emotional	12,3±4,0**	7,5±3,9	41,3±4,4*

(*) $p < 0,05$.
(**) $p < 0,01$.

period) were divided into « emotional » and « non-emotional ». The behavior in the open field was estimated as well as the ability to avoid an acute stress situation (modified Henderson test [12]). Altered balance in the activity of CA systems due to the destruction of CA terminals was followed by increased emotional reactivity and by impaired avoidance behavior (Tab. 4). Both in control « emotional » animals and in rats with destroyed CA terminals tuftsin (200 μg/kg s. c.) improved avoidance performance, thus contributing to the solution of a unsettled « extrapolation » problem: how to escape. This effect makes tuftsin different from other compounds such as amphetamine, haloperidol and clonidine which have different and sometimes opposite effects in animals of different reactivity, especially those whith destroyed CA terminals.

Modulating effect, i.e. an effect depending on the initial state, is characteristic of « modulating peptides ». The mechanism of this phenomenon has not been clarified yet.

Addition of tuftsin to isolated purified rat hypothalamic TH (using the technique of Mineyeva et al. [13]) is followed by inhibition of TH activity (Tab. 5). Tyrosine, the substrate for TH, protected the enzyme from tuftsin inhibitory effect. Fluphenazine, an allosteric regulator of TH activity [14], almost completely eliminated tuftsin's inhibitory effect. This indicates that the interaction between tuftsin and TH is not exerted at the active center of the enzyme, but rather is the result of the changes in conformational requirements. It is probable that the modulating effect of small peptides on CA processes in the CNS also develops at the expense of conformational changes in receptors' « surrounding », in particular, lipid components of the membrane. On the leucocyte membrane, tuftsin molecule by means of electrostatic force interacts with negative groups of sialic acid at the expense of its positively-charged groups [15].

Table 5. – *The effect of tuftsin on isolated rat hypothalamic tyrosine hydroxylase activity in vitro.*

	Reaction rate nmoles · min^{-1} · mg^{-1} protein (M ± m)
Control	355,0±10,0
Tuftsin 10^{-4} M	90,0± 4,5
Tuftsin 10^{-5} M	255,0±15,3
Tuftsin 10^{-4} M *fluphenazine 10^{-4} M	278,0±13,9

The reaction rate was measured by the direct spectrophotometric method. Buffer 0,05 M tris-maleate pH 6,1; 0,11 mM tyrosine; 0,17 mM 6,7 -dimethyl-5,6,7,8-tetrahydropterine; 30 mg protein per ml probe; t° 30 °C.

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A pharmacogenetic approach to the mechanisms of action of opiates

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Summary. – *A number of recent results are reported, showing the existence of strain dependent effects of opiates on behaviour in mice. In fact, opiates administration enhances activity in C57BL/6 mice, a strain in which the analgesic effect is less evident, while decreases it in DBA mice, which are characterized by high levels of analgesia and by a reduced opiate receptors population, as compared with C57 mice. The role played by strain differences* a) *in dopaminergic patterns and* b) *in type and distribution of opiates receptors, in modulating the effects of opiates is also discussed.*

Riassunto. – *Vengono riportati recenti risultati che mostrano come nel topo gli effetti esercitati dagli oppiacei sul comportamento dipendono dal ceppo di roditori preso in esame. Infatti la somministrazione di sostanze stupefacenti aumenta l'attività nei topi appartenenti al ceppo C57BL/6 (C57), in cui l'effetto analgesico è poco evidente, mentre la riduce nel gruppo DBA/2 (DBA) che è caratterizzato da elevati livelli di analgesia e da una popolazione di recettori per gli oppiacei ridotta rispetto ai topi C57. E' anche discusso il ruolo esercitato dalla differenza fra i ceppi* a) *nei meccanismi dopaminergici e* b) *in tipo e distribuzione dei recettori per gli oppiacei, negli effetti comportamentali di queste sostanze.*

Introduction.

Interspecies differences in sensitivity to opiates have been reported by a number of investigators. Among mammals, for example, morphine exerts a narcotic effect in dogs, rabbits, guinea pigs, and rats; excitement follows morphine treatment in cats and horses, while morphine-injected rams, goats, and pigs show an increased motor activity [1]. In humans, the chief features of the morphine picture are a quieting effect and a tendency to sleep; however, failure to obtain sedation has been noted not infrequently.

In recent years, the genetic approach has also proved to be very fruitful in the study of the effects of opiates on behavior in mice. Eriksson and Kiianmaa [2] have analized the susceptibility to morphine addiction in two inbred strains of mice (CBA/Ca and C57BL). In their experiments, mice were given daily morphine injections for 3 weeks. This was followed by a 5-day forcing stage, when the animals were given only aqueous morphine solutions to drink, and a 2-day abstinence period. The mice were then given a free choice between tap water and aqueous morphine solution for 1 week. Morphine consumption was used as the measure of phenotype. The results showed that morphine consumption is higher in the C57BL mice than in the CBA.

In another investigation Gebhart and Mitchell [3] showed the existence of strain differences in the analgesic response to morphine (measured with the hot plate). In their experiments, morphine was found to be 16 times more efficacious in the CF_1 mice than in the CFW strain.

A number of recent studies have demonstrated that C57BL/6J and DBA/2J mice are a very useful tool to study the effects of opiates on behavior. The genetic approach with these strains seems to be a useful method in order to assess which biochemical systems are involved in the various behavioral effects following morphine administration. Differences in behavior and brain chemicals have been demonstrated between these strains in basal conditions [4].

In the present paper are reported the results of a number of studies carried out with the C57 and DBA strains, dealing with the effects of opiates on behavior.

In the first group of experiments, the sensitivity of C57BL/6J and DBA/2J mice to morphine-induced motor activity and analgesia was determined [5]. The locomotor activity was measured with toggle floor boxes, and the effects on the response to painful stimuli with the hot plate method [6] as modified by Goldstein and Sheehan [7]. The results showed that the two strains were clearly different when the morphine-induced « running fit » was considered. A marked increase in locomotor activity was evident in the C57 strain following morphine (or heroin) administration, while no effect or a slight depression appeared when the opiates were injected into the DBA mice (Fig. 1).

When the reaction to painful stimuli was considered, the C57 mice resulted scarcely sensitive to opiates, while in DBA mice the analgesia was very high (Fig. 2). Therefore it was evident that the effects of opiates on both analgesia and running activity were strain-dependent. It was also evident that they were likely to imply two different sites of action, with a negative correlation between the degree of running and the pain obtunding effect.

According to a number of investigations, different models might account for the effects of opiates on running activity and pain appreciation. As previously

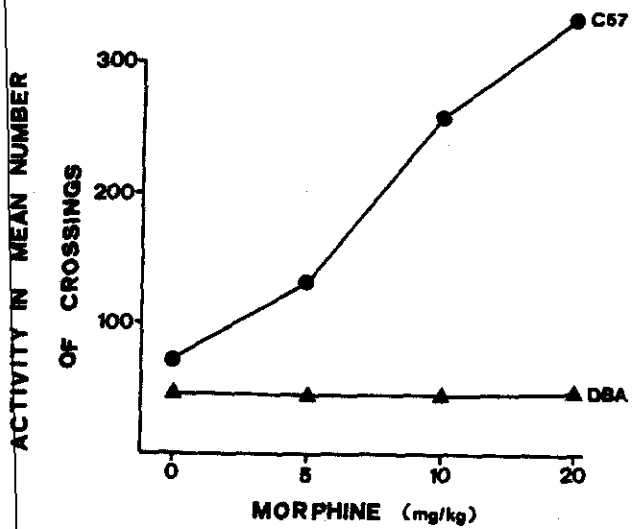

FIG. 1. – Effects of different doses of morphine on the running activity of C57 and DBA mice. Each group consisted of 10 mice. The animals were tested 30 min after the injection of morphine [5].

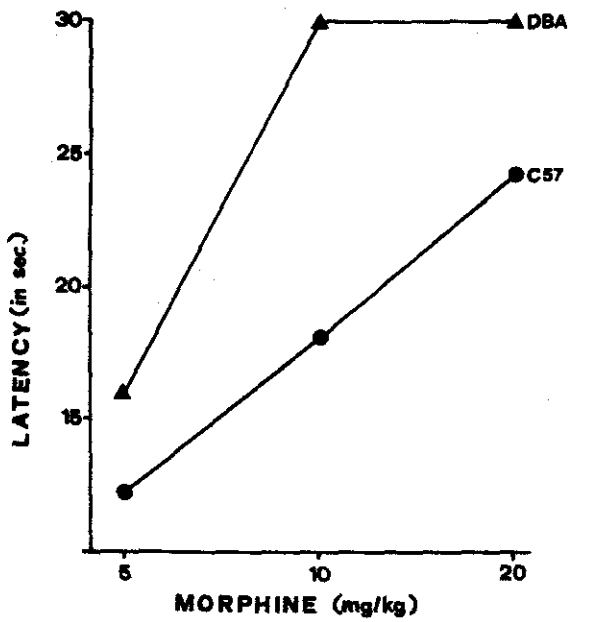

FIG. 2. – Morphine induced analgesia assessed with the hot plate method in C57 and DBA mice. The animals were tested 30 min after the injection with different doses of opiate [5].

mentioned, catecholamine depletion seems to be associated with the running-fit syndrome in mice (and decrease in serotonin levels seems to facilitate it) [8, 9]; cholinergic factors seem, instead, to play an important role in opiate-induced analgesia and abstinence [8, 10]. It may be thus suggested that the biochemical differences existing between C57 and DBA mice might be responsible for the strain-dependent effects of opiates and for the dissociation between the effects of morphine on running and pain-dependent behavior evidenced in our experiments.

Two separate neurochemical patterns seem to be related to the aforementioned effects of morphine. In the C57 mice, whose motor activity is enhanced by opiates, striatal adenylate cyclase is more stimulated by dopamine than in the DBA strain. It has also been demonstrated that striatal dopaminergic neurons are activated mainly in C57 mice; 3-methoxytyramine and cAMP are increased by morphine administration only in the C57 strain [11].

The effect of morphine on DOPAC (3,4 – dihydroxyphenylacetic acid) levels in C57 and DBA mice was also investigated. The drug increased striatal and limbic DOPAC levels in C57 mice by 92 % and 46 % after an injection of 10 mg/kg and 5 mg/kg respectively. On the contrary, morphine injection in DBA mice did not modify DOPAC levels. Since the brain levels of morphine were the same in the two strains, in order to clarify the mechanism of the different effects on dopamine metabolism, opiate receptor function in the same animals was measured. Table I summarizes the results obtained using different radioactive ligands. When (^{3}H)DAME or (^{3}H)Leu-enkephalin were used, a significant difference between the two strains was observed; in fact DBA mice show a lower striatal opiate receptor binding in comparison to C57 mice.

Further kinetic studies indicate that DBA mice possess a reduced opiate receptor population. In fact incubating the tissues with various (^{3}H)DAME or (^{3}H)Leu-enkephalin concentrations to perform a significant reduction in the number of binding sites in DBA mice with respect to C57 mice (171 ± 12 to 102 ± 7 and 157 ± 13 to 92 ± 5 using (^{3}H)DAME or (^{3}H)Leu-enkephalin respectively) was found, while the receptor affinity was similar for the two strains.

These differences are not detectable in the other brain areas studied. On the other hand, using (^{3}H)DHM or (^{3}H)naloxone as radioactive ligands for opiate receptors no significant differences were detected between the two strains of mice either in striatum or in other areas such as brain stem, forebrain, neocortex (Table 1) (12).

Table 1. – *Specific D-[^{3}H] Ala-Met-enkephalin ([^{3}H] DAME), [^{3}H]Leuenkephalin ([^{3}H]Leu) [^{3}H]naloxone ([^{3}H]nal) and [^{3}H]dihydromorphine ([^{3}H]DHM) binding in membrane preparations from the striatum of two different strains of mice* [12].

	C57	DBA
[^{3}H] DAME	94.4±3.0	55.8±1.1*
[^{3}H] Leu	87.6±2.5	45.3±4.1*
[^{3}H] DHM	51.6±3.3	47.1±2.5
[^{3}H] nal	107.6±8.4	99.5±7.3

(*) $p < 0.01$ compared to C57 values in the same area.

The values are expressed in fmol/mg protein and represent the mean ± S.E.M. of 3 different experiments of 5 animals each.

These findings support the hypothesis that enkephalins modulate the function of the striatal DA pathway directly impinging upon DA neurons. In this respect, we may conclude that the so-called dopaminergic effects of narcotics might largely depend on a enkephalinergic-dopaminergic neuronal interaction.

Moreover, the fact that (^{3}H)DHM and (^{3}H)naloxone bindings are similar in the two strains of mice may suggest the presence of different populations of opiate

receptors. It is possible that DBA mice have a higher ratio of type 1/type 2 receptors compared to the C57 strain. In particular, it is possible that the opiate receptors located on striatal dopaminergic terminals, involved in the regulation of morphine effects on dopamine metabolism are, at least in part, type 2 receptors. On the other hand, the type 1 receptors, largely present in DBA mice, may be important in analgesia. This fact is also supported by recent experiments indicating a differential effects of L-aminoacids on C57 and DBA mice [13, 14].

Other pharmacological findings by Sansone et al. [15] also support that differences in dopaminergic patterns in DBA and C57 mice are responsible for morphine-induced hyperactivity in C57 but not in DBA mice. Apomorphine — a dopamine receptor agonist — exerts a biphasic effect on locomotor activity of C57 mice: low doses of the drug reduce motor activity, while high doses elicit hypermotility. In DBA mice, instead, apomorphine depresses locomotor activity over a wide range of doses.

A biphasic action of apomorphine on locomotor activity, similar to that observed in C57 mice, has often been described [16, 17] but locomotor depression at high dosages, as evidenced in DBA mice, has also been reported for other strains of mice [18]. In order to explain the biphasic effect of apomorphine, it has been suggested that low doses of the drug depress locomotor activity by stimulating presynaptic dopamine receptors (autoreceptors) with a consequent reduction in dopamine synthesis. On the contrary, an activation of postsynaptic dopamine receptors would be the cause of the hypermotility produced by the higher doses of apomorphine [16, 17]. This interpretation may be suitable for the effects exerted by apomorphine in C57 mice, but cannot explain the depressant effects always produced by the drug in DBA mice. Strain differences in the behavioral response to apomorphine can perhaps be interpreted on the basis of the two receptor model [19] which was also used to explain the behavioral depression or stimulation induced by different dopamine agonists [20]. The existence of two different dopamine receptors and their different distribution in the brain may also account for the opposite behavioral response to apomorphine exhibited by C57 and DBA mice.

Other findings suggest that dopaminergic mechanisms are responsible to a large extent of morphine-induced running fit: prenatal 60HDA administration results in the abolition of behavioral excitement following acute morphine administration in adult CD1 mice [21].

In general these results indicate that the pharmacogenetic approach provides a useful model for the different opiate receptors involved in the analgesic and motor effects of morphine.

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Effects of oligopeptides on selfstimulation and escape behaviours in the rabbit

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Summary. – *The purpose of the research was to elucidate the influence of intraventricular injections of angiotensin 2, bradikinin and lisylvasopressin on two opposite emotional reactions, selfstimulation and escape in the rabbit. Intraventricular injections of these peptides significantly inhibited the frequency of selfstimulation. Angiotensin 2 and lisylvasopressin significantly increased the latencies of escape responses evoked by electrical stimulation of ventromedial hypothalamic nuclei. The same dose of bradikinin decreased the latencies times.*

Riassunto. – *Nel presente lavoro è stato studiato nel coniglio l'effetto dell'angiotensina 2, della bradikinina e della lisilvasopressina sulle risposte di autostimolazione e sulla reazione di fuga da stimolazione elettrica di specifiche zone cerebrali. I tre peptidi, iniettati nei ventricoli cerebrali, hanno rallentato la frequenza di autostimolazione. La reazione di fuga è stata inibita da angiotensina 2 e lisilvasopressina mentre la bradikinina aveva un effetto opposto.*

Introduction.

The study of the physiological role of various peptides in the organization of different behavioral reactions is of major importance. There is strong evidence that oligopeptides participate in the processes involved in learning, memory, sleep and motivational excitation [1–5]. Researches on endogenous oligopeptides, angiotensins and kinins are of particular interest since these peptides are involved in the regulatory processes of the homeostatic functions in the organism [6–9].

In addition, the influence of oligopeptides on emotional reactions is still to be investigated. The purpose of our research is to elucidate the influence of intraventricular injections of angiotensin 2, bradikinin, lisylvasopressin on two opposite emotional reactions, such as selfstimulation and escape. Since there is evidence of rapid inactivation of peptides in the organism under systemic injections [10], it was decided to use intraventricular injections.

Methods.

Experiments were performed on 37 male rabbits under free-moving conditions. Reactions of selfstimulation and escape were produced by electrical stimulation via 2 electrodes implanted into the lateral hypothalamic region and ventromedial nuclei respectively. For injecting chemicals into the brain lateral ventricles special cannulas were used. We applied current of the following parameters: frequency 100hz, impulse duration 1,4 msec, tension 1–4 volts.

Hypothalamic stimulation lasted 3 sec, whereas duration of self-stimulatory impulses was 0,3 sec. Selfstimulation behavior was measured on the basis of the frequency of lever pressings during 30 sec. This behavior was measured for 3 hours. Control of selfstimulation frequency (100 %) was assessed during 30 minutes. All the variations of this frequency were expressed as percent variations of control. Latency to escape was measured in all animals. Angiotensin 2, bradikinin or lisylvasopressin were injected into the ventricles 30 minutes after recording of control selfstimulation behavior and observations of escape reactions latencies (for three times) in animals. The chemicals were used at the dose levels of 5, 50, 150 ng, dissolved in 3 µl. of distilled water. Saline or distilled water was injected in control experiments.

Results and discussion.

In ten control rabbits mean frequency of self stimulation and escape latencies remained constant during 3 hours of observation. The changes of selfstimulation within 3 hours after angiotensin 2, bradikinin, lisyl-vasopressin injections are represented on Fig. 1. The following dose-dependent effects were discovered. Intraventricular injections of angiotensin 2 at the dose of 5 ng caused within 40 minutes inhibition of selfstimulation in 4 rabbits out of 6. The mean frequency of selfstimulation in this group became 62,3 % lower by this time in comparison to the initial level. At the dose of 50 ng angiotensin inhibited self stimulation in 6 rabbits between 20 and 70 minutes after the injection. Angiotensin 2 at the dose of 150 ng caused full inhibition of selfstimulation in all 12 rabbits during 40 minutes (Fig. 1, *a*).

It should be noted that at the same doses injections of bradikinin caused complete disappearance of selfstimulation in all 12 rabbits of this group within the following intervals: 5 ng in 100 min., 50 ng in 80 min., and 150 ng in 60 min (Fig. 1, *b*).

Intraventricular injections of lisyl–vasopressin at the dose of 150 ng decreased the frequency of selfstimulation in 75 % of animals. The highest inhibition of selfstimulation behavior (close to complete inhibition) was observed only 150 ± 30 minutes after the peptide injection; 25 % of the animals didn't change the frequency of lever pressing after lisyl–vasopressin injection. At the dose of 5 and 50 ng this peptide induced a complete suppression of selfstimulation for 3 hours. It should be noted that for 3 hours after the injection the lever pressing frequency was still significantly decreased: of about 70 % (50 ng) and of 50 % (5 ng) in comparison with the initial values (Fig. 1, *c*).

Thus, intraventricular injections of these peptides significantly inhibited the frequency of selfstimulation in rabbits. Changes in latencies of escape responses were different depending on the peptide considered and on the time elapsed after the injection. Bradikinin (150 ng) induced a reduction of the latencies by the end of the 1st hour (from 7,5 ± 0,2 sec. to 2,3 ± ± 0,1 sec.) in 11 rabbits out of 12. The same dose of lisyl–vasopressin induced an inhibition of escape behavior. In 40 % of the animals a 2–3 time increase in latency of the escape reaction was evident after the injection; this increase was still present after 1–1,5 hours. In 20 % of animals the highest latencies appeared 20–40 minutes after lisyl–vasopressin injection, no complete recovery of escape responses was noticed in the following 3 hours. In 40 % of animals no changes of the latency of escape reactions were evident (Fig. 2, *b*).

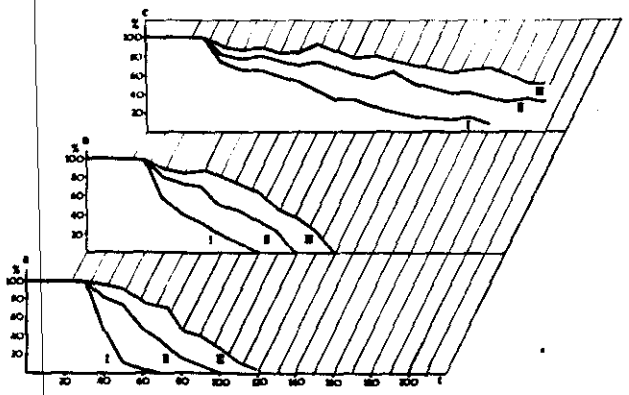

FIG. 1. – Dose dependent changes of selfstimulation under intraventricular angiotensin 2 injection (a), bradikinin injection (b), lisyl–vasopressin injection (c). 1 = 150 ng; 2 = 50 ng; 3 = 5 ng Abscissa = time in minutes. Ordinate = frequency of selfstimulation (in % of initial value).

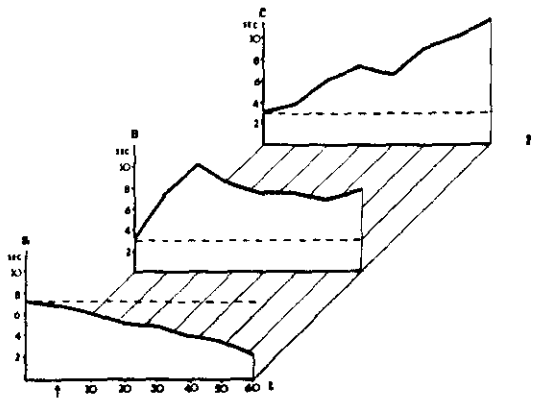

FIG. 2. – Changes in latencies of rabbit's escape reaction under intraventricular bradikinin injection (a), lisyl–vasopressin (b) and angiotensin 2 (c) (150 ng). Abscissa = time in minutes. Ordinate = latencies escape reaction in sec. The arrow indicates the injection.

Intraventricular injections of angiotensin 2 at the dose of 150 ng increased the latencies from 2,9 ± 0,4 sec. to 12 ± 2 sec. in 10 animals (Fig. 2, *c*). The highest values were obtained by the end of the first hour after the injection. In one case only the latencies changes of escape reaction were not significant after angiotensin 2 injection.

Thus, intraventricular injections of angiotensin 2 and lisyl–vasopressin significantly increased the latencies of escape responses, evoked by electrical stimulation of ventromedial hypothalamic nuclei. The same dose of bradikinin decreased the latencies times.

These results confirm the fact that intraventricular injections of these peptides affect the emotional reactions of animals. This is proved by a number of researches [6, 11, 12]. The central action of angiotensin 2 may also be determined by its direct interaction with specific brain receptors [13, 14] as well as by influence on brain transmitters [15, 16] such as serotonin [17] or acetylcholine [18] whose turnover is modified by angiotensin 2. Unlike angiotensin 2, the effects of vasopressin on the emotional reactions may be different and due to the direct influence of this peptide on protein hypothalamic metabolic processes [19].

Intraventricular bradikinin injections influencing bradikinin sensitive hypothalamic structures are likely to have antagonist effects on norepinephrine, angiotensin and vasopressin. This effect was demonstrated at the level of microcirculation. The injection of these substances produces diversified effects on negative emotional reactions of animals, *i.e.* escape reaction.

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