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The Role of the Nucleus Accumbens and Nigrostriatum in Enkephalin-Induced Myoclonus

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DZOLJIC, M. R. AND A. L. V.D. POEL-HEISTERKAMP. *The role of the nucleus accumbens and nigrostriatum in enkephalin-induced myoclonus.* PHARMAC. BIOCHEM. BEHAV. 13(1) 103–106, 1980.—Local administration of the enkephalin-analog, D-Ala^2-Met^5-enkephalinamide (DALA) into the nucleus accumbens and caudate nucleus of the rat resulted in myoclonic contractions (MC) of the submandibular muscles and epileptic discharges in the electrocorticogram. These phenomena were blocked by naloxone but not by haloperidol. Similar administration of DALA into the substantia nigra compacta failed to produce MC. A possible involvement of endogenous opiates in the nucleus accumbens and striatum is suggested in the pathogenesis of some types of myoclonus.

Enkephalin Myoclonus D-Ala^2-Met^5-enkephalinamide Nucleus accumbens Naloxone Caudate nucleus Haloperidol Substantia nigra compacta

RECENTLY, we found that the enkephalin analog D-Ala^2-Met^5-enkephalinamide (DALA), injected intraventricularly, induces myoclonic contractions (MC) in the submandibular muscles of rat [8]. This effect was ascribed to the enkephalin-induced activation of the "inhibitory" types of dopamine receptors. Similarly, recent data indicate that the locomotor activity triggered by ergometrine administration into the nucleus (n.) accumbens is due to the modulation of the activity of distinct types of dopaminergic receptors which are unequally distributed between the neostriatal and mesolimbic dopaminergic structures of rat brain [5,6]. These and other experiments [23] provide evidence that the mesolimbic dopamine system, with cell bodies in the A 10 group and nerve terminals in the n.accumbens and tuberculum olfactorium, is functionally different from the nigrostriatal dopamine system. In addition to dopaminergic receptors, the n.accumbens and nigrostriatum also contain relatively high concentrations of opiate receptors [12,20] and both structures were involved in the opiate-induced motor activity [13,21].

However, in spite of intensive research work, the pathophysiology of myoclonus is not yet known. Some patient and animal studies have provided evidence that dysregulation of serotonin activity might induce body jerks [14,15]. Other experimental data implicate inhibition of the action of gamma-aminobutyric acid in the caudate nucleus as a possible mechanism of myoclonus [23].

The aim of the present study was to clarify the roles of different anatomical structures, particularly those of the mesolimbic and nigrostriatal dopaminergic system, in the enkephalin-induced myoclonic activity.

We report here that microinjection of DALA into the n.accumbens and caudate n., but not injection into the substantia nigra compacta, resulted in myoclonic activity of the submandibular muscles of the rat and in an epileptic electrocortical (ECoG) pattern.

METHOD

Local Microinjections

Male Wistar rats (175–250 g) were used. Under urethane anaesthesia (1,2 g/kg) stainless steel cannulae were implanted bilaterally by means of a stereotaxic instrument. The coordinates of the tips of the injection cannulae were: A 9.4, H 2.4, L 1.2 for the n.accumbens; A 2.2, H 2.2, L 2.0 for the n.caudatus and A 9.4, H 2.0, L 2.0 for the substantia nigra compacta, according to the stereotaxic atlas of the rat brain of König and Klippel [16]. The cannulae were made from 27 ga stainless steel tubing and attached to the skull with dental acryllic cement. Injections were made by means of 5 μl micrometer-driven syringe (Hamilton) with a needle which extended into the brain tissue 1 mm below the tip of the cannula. The cannulae placed in the caudate n. and n.accumbens were inserted from the lateral side at an angle of 5° and 10°, respectively, in order to avoid the ventricles. The substances and control injections were injected bilaterally in a volume of 0.2–0.5 μl.

ECoG and Electromyographic (EMG) Recording

The ECoG recording and details related to the implantation technique and apparatus used have been described earlier [7].

After trachea cannulation by the method of Bieger *et al.*

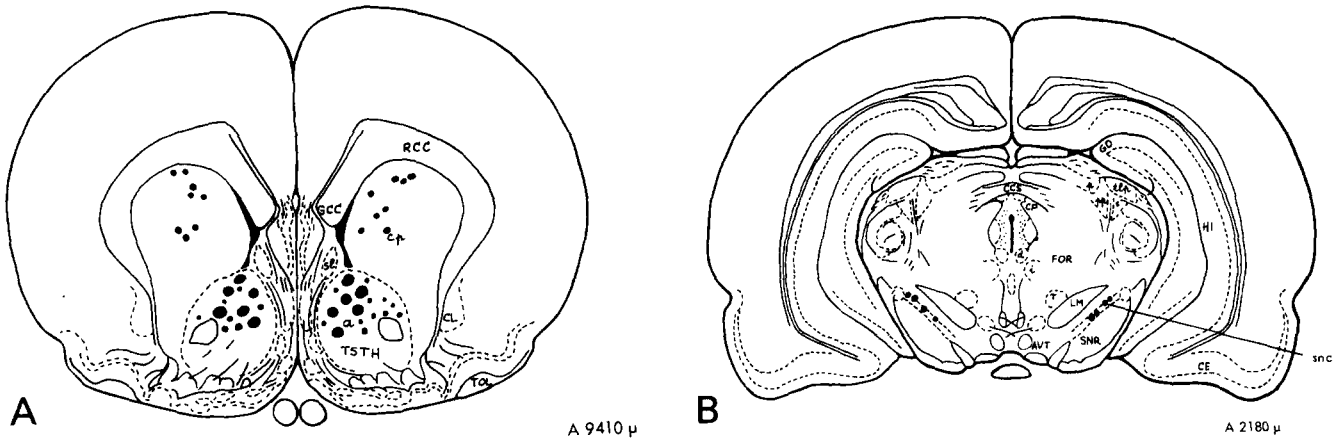

FIG. 1A,B. Frontal sections of the rat brain according to the atlas of König and Klippel [16]. Location of dye spots in the caudate-putamen (cp), nucleus accumbens (a) and substantia nigra-zona compacta (snc) indicate sites of injections. Each spot represents 1–2 experiments (small spot) or 3–8 experiments (animals-larger spot).

[2], the spontaneous or drug induced myoclonic activity of inframandibular muscles (superior belly of digastric and mylohyoid muscles) was recorded electromyographically by means of a polygraph Grass Model 7. We observed that EMG spikes representing the MC were of different voltage amplitude. Therefore, in the "results" only those spikes which exceed 50 μV were evaluated.

Drugs

Peptides and other drugs were dissolved in artificial fluid (CSF) immediately before intracerebral (ICB) injection. The

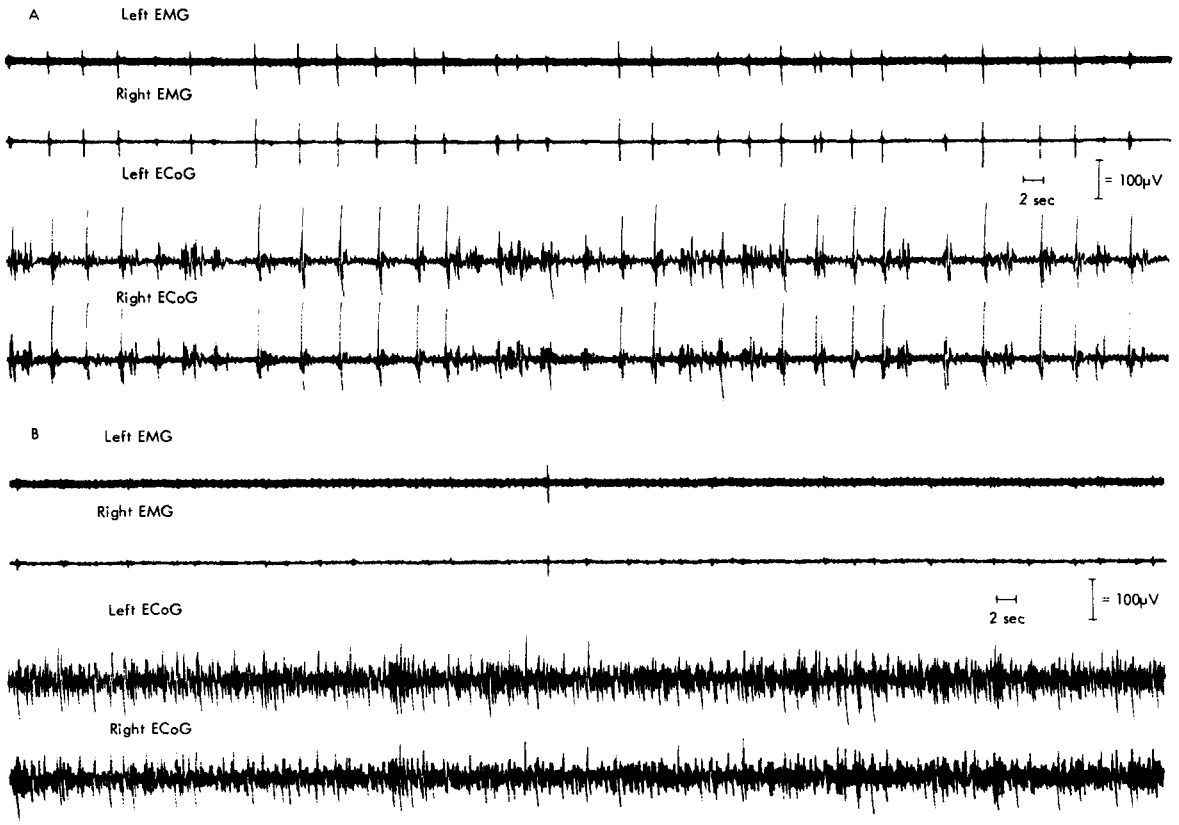

FIG. 2. Electromyogram (EMG) of the submandibular muscle (mylohyoid muscle) and electrocorticogram (ECoG) between the frontal and parietal electrodes of an anaesthetized rat (urethane, 1.2 g/kg). EMG and ECoG after 2 min (A) and 48 min (B) of injection of DALA (2 μg) into the n.accumbens. Note the temporal association between the submandibular MC and the epileptic ECoG spike discharges.

control injections consisted of the vehicle alone. The drug dosages are expressed in terms of their salts.

Histological Procedures

At the end of the experiment 0.5 μl of silver nitrate (20%) was injected through the cannula into the brain. The brain was removed and placed in a 10% Formalin solution for several days. Subsequently, the brain was dehydrated and embedded in paraffin and serial 10 μm thick sections were cut and stained with cresyl violet. The position of the needle track were marked as shown in Fig. 1.A,B.

RESULTS

Bilateral application of DALA (0.2–16 μg) to the n.accumbens or n.caudatus induced a dose-related increase in MC in the submandibular muscles within a few minutes after injection of the drug. The increase in MC ranged from 4 to 68/min. Parallel with the MC, recorded as EMG spikes, the ECoG phenomena we also followed. Analysis of both recordings demonstrated a temporal association between the EMG and ECoG spike discharges (Fig. 2). Both phenomena lasted for about 10–30 min. Similar administration of DALA (0.5–16 μg) into the substantia nigra-pars compacta induced spike discharges in the ECoG but not MC.

The DALA-induced MC and ECoG spike discharges were antagonized by intraperitoneal administration of naloxone (0.5–2 mg/kg 15–30 min before DALA administration, Fig. 3). However, neither local application of haloperidol (0.01–5 μg) to the n.accumbens or n.caudatus, nor the IP administration of this drug (0.1–4.0 mg/kg 60 min prior to local administration of DALA) significantly influenced the DALA-induced EMG or ECoG pattern.

DISCUSSION

These data suggest that activation of the stereospecific opiate receptors sensitive to naloxone in the n.accumbens and n.caudatus, results in MC of submandibular muscles in the rat. Furthermore, this study supports the role of the caudate nucleus in the generation of MC, as suggested earlier [23], but also indicates an involvement of the forebrain, particularly the n.accumbens, in the myoclonic phenomena. However, in spite of the evidence that opiate receptors are located on dopamine cell bodies in the substantia nigra [20], the local administration of DALA into this structure did not produce MC. Evidently, this is a clear indication that in some regions of the brain exists a dissociation between motor and ECoG phenomena induced by enkephalins.

Of particular interest, however, is the temporal association of MC and the epileptic spike discharges observed in the ECoG. An epileptiform ECoG has already been demonstrated in rats following intracerebral or intraventricular administration of Leu^5-enkephalin, Met^5-enkephalin, β-endorphin and DALA [9, 11, 24]. With regard to the behavioural response, it has been recently reported that local injection of DALA into the n.accumbens produces an increase in locomotor activity [21]. Evidently, in the rat forebrain, the opiates produce excitatory effects, which under certain circumstances may appear in the form of myoclonus. Therefore, the statement that β-endorphin induces nonconvulsive limbic seizures [11] should not be generalized and the possibility of involvement of the endogenous opiates in convulsive seizures, particularly of myoclonic types, has also to be considered.

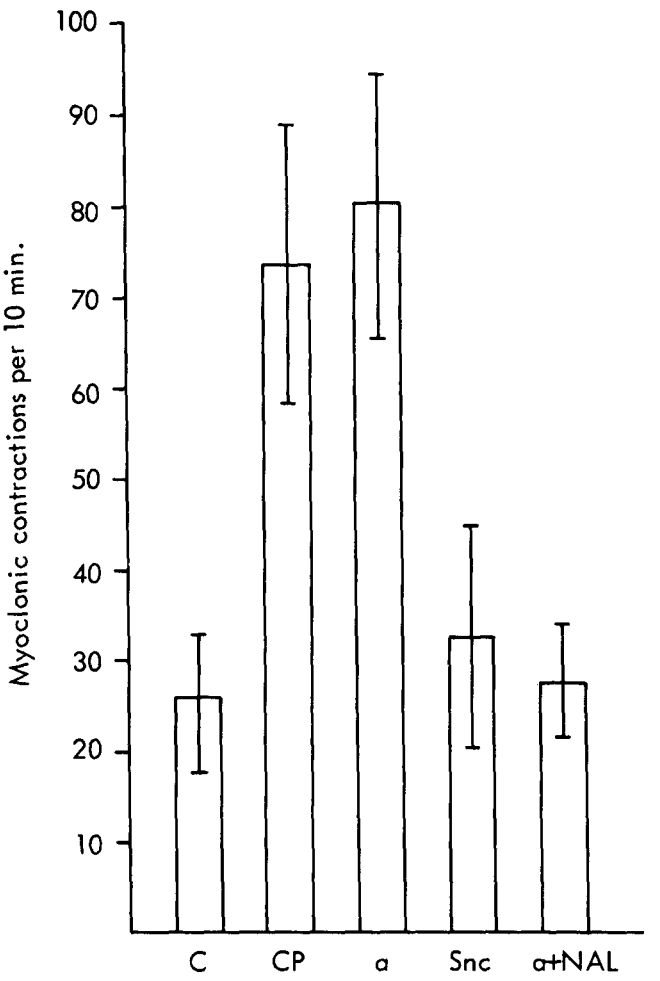

FIG. 3. Myoclonic contractions (MC) after bilateral application of 2 μg DALA to the substantia nigra-pars compacta (Snc, N=6), caudate nucleus (CP, N=8) and nucleus accumbens (a, N=36) of the anaesthetized rats. DALA was injected into the nucleus accumbens 15 min after naloxone (a+NAL, 2 mg/kg, intraperitoneally). In control animals divided in three equal groups (6/per group) for each anatomical region, CSF was injected. The results between groups were not essentially different, therefore, the responses of all control animals are expressed in form of one column (C). The vertical line indicates the number of myoclonic contractions obtained during the first 10 min after local administration of DALA or CSF. Vertical bars indicate SEM.

Considerable evidence supports a role of the dopaminergic system in the central effects of opiates [13,17], including the data on increased striatal turnover of dopamine after intraventricular administration of β-endorphin and DALA [1,19]. The n.accumbens is one of the important projection areas of the dopaminergic cell bodies into the ventral tegmental region and there is evidence that opiates may produce excitation by activating dopaminergic neurons [3,4]. However, failure of haloperidol to antagonize the MC in the n.accumbens and n.caudatus indicates that this excitatory effect of DALA should be dissociated from a direct action on the dopaminergic receptors sensitive to haloperidol. Similarly, haloperidol failed to decrease the MC after intraventricular administration of DALA and the ECoG sei-

zure phenomena following administration of β-endorphin in the rat [8,11]. Evidently, further studies are necessary to define the precise role of the dopaminergic system and other neurotransmitters, particularly GABA, in enkephalin-induced myoclonus.

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