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On the Possible Role of Excitatory Amino Acids in the Striatum in Mediating Morphine-Induced Muscular Rigidity

LECHOSLAW TURSKI,[1] URSULA HAVEMANN[2] AND KLAUS KUSCHINSKY

Department of Biochemical Pharmacology, Max Planck Institute for Experimental Medicine D-3400 Göttingen, Federal Republic of Germany

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TURSKI, L., U. HAVEMANN AND K. KUSCHINSKY. *On the possible role of excitatory amino acids in the striatum in mediating morphine-induced muscular rigidity*. PHARMAC. BIOCHEM. BEHAV. **17**(4) 715–719, 1982.—The possible role of excitatory amino acids in the striatum in mediating tonic activity in the electromyogram (EMG) was studied. Glutamate diethylester (GDEE) (100–400 nmoles) induced a tonic activity in the EMG in a dose-dependent way when injected into the striatum. This effect was well antagonized by intrastriatal injection of quisqualic acid (5 and 25 nmoles), less by kainic acid (5 nmoles) and not significantly by N-methyl-D-aspartate (NMDA) (30 nmoles). Systemic administration of naloxone (2 mg/kg IP) did not inhibit the GDEE-induced activity in the EMG. The tonic activity in the EMG, induced by systemic administration of morphine (15 mg/kg IP) was not significantly influenced by injection of GDEE (200 nmoles) into the striatum, but was first decreased and then slightly enhanced by intrastriatal injection of quisqualic acid (25 and 50 nmoles), not affected by kainic acid (5 nmoles) and first slightly decreased and then strongly enhanced by NMDA (15 and 30 nmoles). Injection of kainic acid (5 nmoles), quisqualic acid (5 or 25 nmoles) or NMDA (30 nmoles) alone into the striatum did not produce any tonic activity in the EMG. Our results support the assumption that quisqualic acid, kainic acid and NMDA react with different types of receptors for excitatory amino acids in the striatum. Both quisqualicacid and NMDA showed a biphasic action, whereas kainic acid was ineffective. Furthermore, the activity in the EMG induced by morphine might be at least partly due to a functional antagonism of morphine against glutamate in striatal neurons.

Morphine Muscular rigidity Electromyogram Striatum Quisqualic acid Kainic acid N-Methyl-D-aspartate Glutamic acid diethylester

SYSTEMIC administration of morphine induces a muscular rigidity in rats, which can be recorded from the gastrocnemius-soleus (GS)-muscle as a tonic activity in the electromyogram (EMG) [23]. The muscular rigidity is one of the prominent signs of opioid-induced "catatonia" and opioid receptors in the head of the caudate nucleus are believed to mediate it [10, 11, 12]. The striatum (caudate nucleus + putamen, which are not clearly separated in rats) receives a major input from pyramidal cells of the layer V of the cerebral cortex [13]. A number of studies suggested that glutamate is the neurotransmitter of this cortico-striatal pathway [3, 5, 15, 17, 21]. It seems likely that this pathway influences the function of the striatum in a crucial way. Selective lesion studies suggested that some opioid receptors are located on terminals of this glutamergic pathway [2,22], although the existence of these receptors was doubted by Murrin *et al.* [20]. Since opioid receptors located presynaptically on nerve terminals decrease the release of many neurotransmitters, the hypothesis seems plausible that morphine might produce the muscular rigidity by inhibiting the release of glutamate in the striatum. Alternatively, the rigidity might be at least in part due to a functional antagonism of opioids vs glutamate on single striatal neurons, as found by Frederickson and Norris [6] and Fry *et al.* [7]. In both cases, a blockade of striatal glutamate receptors should more or less mimick the morphine-induced muscular rigidity and enhance this effect. Agonists of glutamate receptors, on the other hand, should antagonize muscular rigidity produced by morphine. In order to evaluate this assumption, either the relatively selective antagonist, glutamate diethylester (GDEE) [8,9] or drugs activating more or less specifically glutamate and other excitatory amino acid receptors [19,24]: quisqualate, N-methyl-D-aspartate (NMDA) and kainic acid were injected into the striatum and their effects on the morphine-induced muscular rigidity were studied.

METHOD

Animals

Male Wistar rats (TNO/W 70, F. Winkelmann, Borchen, FRG) weighing 200–230 g were used throughout the experiment. Prior and after surgery the animals were kept on standard light-dark cycle (lights on 0600–1800) and received food and water ad lib. The rats were assigned to experi-

[1]On leave from Department of Pharmacology, Institute of Clinical Pathology, Medical School, Lublin, Poland.
[2]To whom correspondence should be addressed.

mental groups by means of a completely randomized schedule. To determine the subsequent time of behavioral testing for different treatment groups, a completely randomized schedule was also employed. Experimental groups consisted of 6–17 animals.

Implantation of Guide-Cannulas

For intrastriatal injection a permanent guide cannula was implanted unilaterally under pentobarbital (50 mg/kg IP) anesthesia applying the coordinates given by Fifková and Maršala [4]. AP: −1.25; L: 2.6; V: 4.5, for details of the method see Havemann *et al.* [12]. After the implantation the animals were housed individually in their cages and 5–7 days later the experiment was performed. Each animal was used once only.

Drugs and Administration Procedure

L-Glutamic acid diethylester (Sigma, St. Louis, MO), kainic acid (Sigma, St. Louis, MO) and M-methyl-D-aspartate monohydrate (Tocris Chemicals, Roebuck Lane, England) were dissolved in saline and the pH was adjusted to 7.4 with 0.8 N NaOH. The drugs were slowly injected into the head of the nucleus caudatus (volume 1 μl). The intrastriatal injection lasted for 5 min (0.2 μl/min) and subsequently the injection cannula was maintained in position for a further 2.5 min. Control rats received the same volume of solvent by the same procedure. Morphine hydrochloride (Merck, Darmstadt, FRG) and naloxone hydrochloride (Endo Labs., Garden City, NY) were dissolved in saline and administered intraperitoneally in a volume of 0.15 ml/100 g body wt. All doses refer to the free base.

Measurement of the Activity in the Electromyogram

The activity in the EMG was recorded from the GS muscle of the hindlimb of non-anesthetized rats sitting in a special cage, as described elsewhere [12]. The signals under investigation were amplified, bandpass-filtered (5 Hz–10 kHz), rectified and fed into an integrator, which was automatically reset after a preset voltage was reached. The reciprocal of the reset-time of the integrator was the measure of the activity in the EMG. The activity was recorded continuously and the values of the mean activities of 5 min intervals were calculated. In contrast to our previous studies we now recorded the activity in the EMG by pairs of percutaneously inserted, teflon insulated, stainless steel fine wire electrodes (Cooner Wire, AS 632 SS). These electrodes, in comparison with bipolar needle electrodes used in the previous studies, had the advantage of sampling activity changes in the EMG from a greater number of motor units in the muscle under investigation.

Histology

After completion of each electromyographical experiment the location of the injection site was verified histologically by cresyl violet stained serial sections.

Statistical Analysis

The data collected from the electromyographical experiments were evaluated by means of non-parametric Mann-Whitney U test.

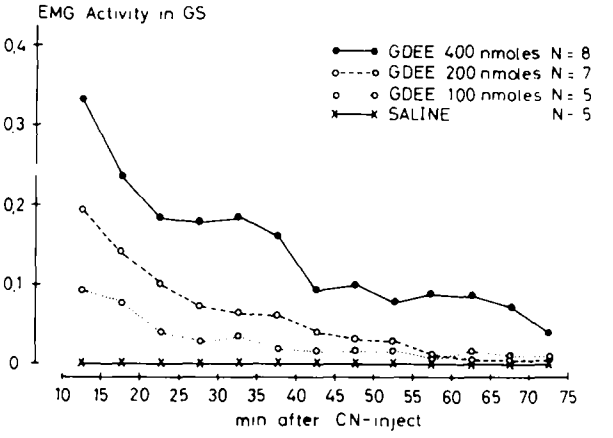

FIG. 1. Time-course of the activity in the EMG induced by intrastriatal injection of various doses of GDEE or of saline into the striatum (caudate nucleus=CN). Abscissa: time (min) after beginning of the injection; ordinate: EMG activity in the gastrocnemius-soleus (GS) muscle. Median values.

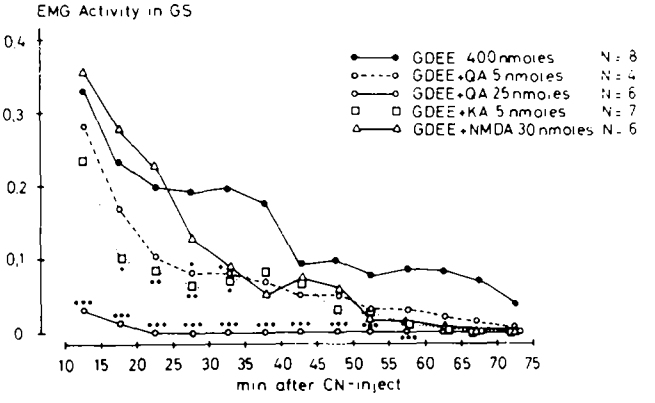

FIG. 2. Time-course of the activity in the EMG induced by intrastriatal injection of GDEE in combination with quisqualic acid (QA), kainic acid (KA) or N-methyl-D-aspartate (NMDA). For further explanations, see Fig. 1.

RESULTS

An injection of 400 nmoles of glutamate diethylester (GDEE) induced a tonic activity in the EMG, which for technical reasons could be recorded not earlier than 10 min after beginning of the intrastriatal injection, since the injection procedure had to be performed outside the cage used for recording of the EMG. The activity in the EMG decreased during the subsequent 60 min and almost completely disappeared 75 min after beginning of the injection (Fig. 1).

This effect was dose-dependent, since the effect of 200 nmoles was less pronounced and 100 nmoles produced a very weak and short-lasting effect only. Saline injections were ineffective. The activity in the EMG induced by GDEE was almost completely suppressed by a simultaneous injection of 25 nmoles of quisqualic acid (Fig. 2), whereas 5 nmoles of quisqualic acid as well as 5 nmoles of kainic acid

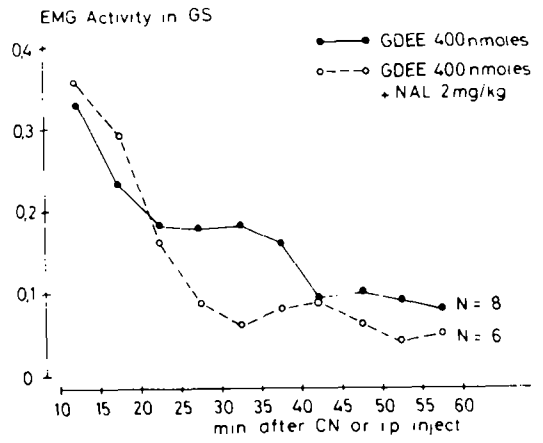

FIG. 3. Time-course of the activity in the EMG induced by intrastriatal injection of GDEE, injected immediately after systemic administration of naloxone (NAL) (2 mg/kg IP). For further explanations, see Fig. 1.

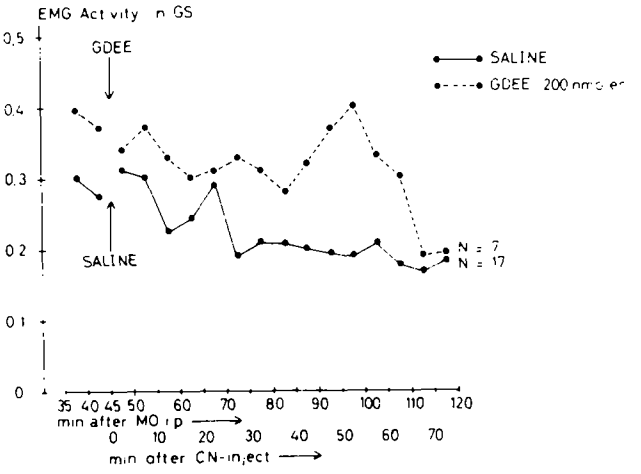

FIG. 4. Effect of an intrastriatal injection of GDEE or of saline on the activity in the EMG induced by systemic administration of morphine (MO) (15 mg/kg IP). The intrastriatal injection was performed 45 min after the systemic administration of morphine. For further explanations, see Fig. 1.

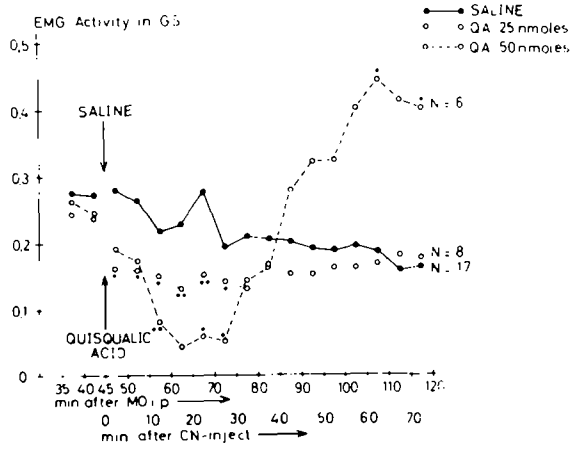

FIG. 5. Effect of an intrastriatal injection of quisqualic acid (QA) or of saline on the activity in the EMG induced by systemic administration of morphine (MO) (15 mg/kg IP). The intrastriatal injection was performed 45 min after the systemic administration of morphine. For further explanations, see Fig. 1. Significances: $*\alpha<0.05$; $**\alpha<0.01$, compared with saline controls at the same time (Mann-Whitney U test).

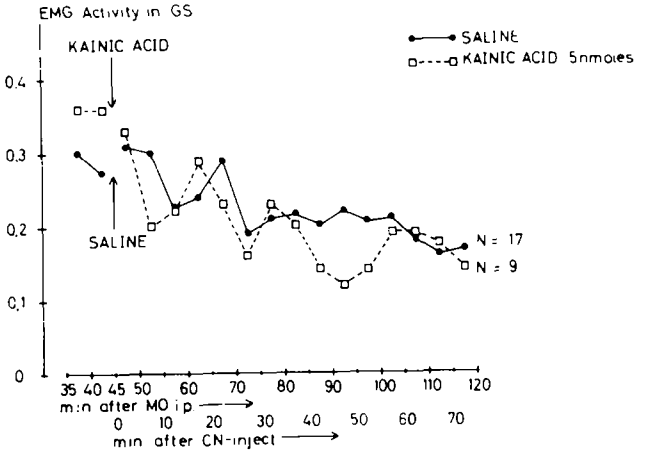

FIG. 6. Effect of an intrastriatal injection of kainic acid or of saline on the activity in the EMG induced by systemic administration of morphine (MO) (15 mg/kg IP). The intrastriatal injection was performed 45 min after the systemic administration of morphine. For further explanations, see Fig. 1.

were less effective, although the effect of kainic acid was significant, and 30 nmoles of N-methyl-D-aspartate induced only a very slight, if any, inhibition. Systemic administration of naloxone (2 mg/kg IP) did not inhibit the GDEE-induced activity in the EMG (Fig. 3). Injection of kainic acid (5 nmoles), quisqualic acid (5 or 25 nmoles) or NMDA (30 nmoles) alone into the striatum did not produce any tonic activity in the EMG. Systemic administration of naloxone (2 mg/kg IP) was also ineffective.

In the next series of experiments, morphine was injected systemically (15 mg/kg IP) and the activity in the EMG was recorded immediately before the intrastriatal injection of the drug, which occurred 45 min after the administration of morphine, when the plateau of the morphine effect was obtained. In this series, the rats were akinetic and could be easily injected intrastriatally during recording of the EMG. GDEE (200 mnoles) did not significantly enhance the morphine-induced activity in the EMG (Fig. 4). Intrastriatal injection of quisqualic acid antagonized this activity for about 30 min in a dose-dependent way (Fig. 5) and after this time, the higher dose of quisqualic acid seemed to enhance the EMG activity. In contrast, intrastriatal injection of kainic acid did not influence the morphine-induced activity in the EMG (Fig. 6). NMDA in the lower dose (15 nmoles) slightly inhib-

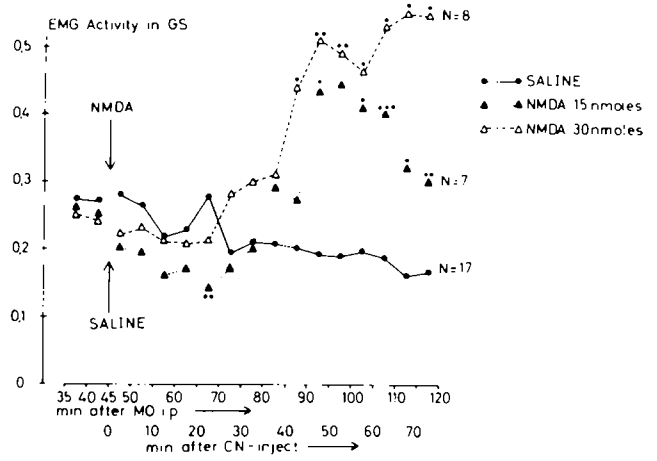

FIG. 7. Effect of an intrastriatal injection of N-methyl-D-aspartate (NMDA) or of saline on the activity in the EMG induced by systemic administration of morphine (MO) (15 mg/kg IP). The intrastriatal injection was performed 45 min after the systemic administration of morphine. For further explanations, see Fig. 1. Significances: $*\alpha<0.05$; $**\alpha<0.01$; $***\alpha<0.002$, compared with saline controls at the same time (Mann-Whitney U test).

ited the morphine-induced activity in the EMG for a short time (Fig. 7) and in a second phase beginning about 40 min after its injection enhanced the morphine-induced activity in the EMG. The higher dose of NMDA (30 nmoles) did not inhibit the activity in the EMG at the beginning, but after a delay of about 30 min enhanced the activity in the EMG even in a more pronounced way than the lower dose.

DISCUSSION

Intrastriatal injections of glutamate diethylester (GDEE), which blocks the excitatory action of L-glutamate [14,21] and competes with its binding to brain tissue [1], induced a tonic activity in the electromyogram (EMG). This effect of GDEE cannot be due to a direct action on opioid receptors, since administration of naloxone did not influence the effect of GDEE, and therefore it must be due to an action "downstream" of the site of action of opioids. Quisqualic acid (5 nmoles) and kainic acid (5 nmoles) antagonized the GDEE-induced activity in the EMG to a similar degree, whereas a dose of N-methyl-D-aspartate as high as 30 nmoles only slightly antagonized the GDEE effect. The first two observations seem to be in good agreement with in vitro observations of Luini *et al.* [16], who found that kainic acid and quisqualic acid were nearly equipotent in inducing $^{22}Na^{+}$-efflux from preloaded striatal slices. In contrast, however, NMDA was much more potent in these slices. Our results present no clear evidence, however, that the effect of GDEE is the result of a specific blockade of glutamate receptors; in our system, GDEE, in addition, might block receptors for kainic acid, which appear to be distinct from those activated by glutamic acid [18,24]. In spite of these problems about specificity at glutamate receptors, these results seem to be in agreement with the assumption that morphine might induce the tonic activity in the EMG at least partly by a decrease of glutamergic neurotransmission in the striatum, e.g., by a decrease of glutamate release or a postsynaptic antagonism of opioids vs glutamic acid. Accordingly, quisqualic acid, in the dose of 25 nmoles, slightly antagonized the morphine-induced activity in the EMG, while the same dose of quisqualic acid almost completely inhibited the activity in the EMG induced by GDEE. A higher dose of quisqualic acid was necessary to antagonize the morphine-induced activity in the EMG. The latter finding does not support the assumption that the morphine-induced activity in the EMG is due to a decrease in release of glutamate from cortico-striatal neurons, since in this case the morphine-induced activity in the EMG should be antagonized by quisqualic acid more easily than that induced by the blocker of glutamate receptors, GDEE. Our finding seems to be, however, in a good agreement with the assumption that the effect of morphine might be due to a functional antagonism against glutamate on striatal neurons, as was found by Fry *et al.* [7] using microiontophoretic techniques. If such a functional antagonism exists, GDEE might not necessarily enhance the activity in the EMG produced by morphine.

NMDA, on the other hand, was more potent than quisqualate in inducing the delayed enhancement of the activity in the EMG produced by morphine. This observation might be explained by the assumption [18,19] that quisqualic acid activates another type of receptors than NMDA. The receptors with preferences for the latter drug might be located on different neurones from those with preference for quisqualic acid, which might well explain the different effects of both substances on morphine-induced activity in the EMG. The delay in the onset of the enhancement might be due to the time necessary for a diffusion from the injection site to those neurons within the striatum equipped with receptors with preference for NMDA or to biphasic changes at the neuronal level. Kainic acid did not significantly influence the morphine-induced activity in the EMG. This might be explained by the hypothesis [18,24] that kainic acid reacts preferentially with a third type of excitatory amino acid receptors.

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