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Morphine-induced catalepsy is augmented by NMDA receptor antagonists, but is partially attenuated by an AMPA receptor antagonist

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Abstract

High doses of morphine produce a state of behavioural inactivity and muscular rigidity. This type of 'catalepsy' is clearly different from the state which is produced by the administration of neuroleptics, e.g. haloperidol. While haloperidol-induced catalepsy can easily be antagonised by NMDA receptor antagonists, there has been a report that the non-competitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) potentiates morphine-induced catalepsy. The aim of this study was to further examine the role of glutamate receptors in the mediation of morphine-induced catalepsy. To this end we coadministered morphine (20, 40, 60 mg/kg i.p.) with MK-801 (0.1 and 0.3 mg/kg i.p.), the competitive NMDA receptor antagonist DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentoic acid (CGP 37849) (2 and 6 mg/kg i.p.), or 1-(4-aminophenyl)-4-methyl-7,8-methylen-dioxy-5*H*-2,3-benzodiazepine (GYKI 52466) (2 and 4 mg/kg), an antagonist of the AMPA type of glutamate receptors, respectively. The degree of catalepsy was assessed using two different methods, the 'bar/podium/grid' test which is commonly used to measure neuroleptic-induced catalepsy, and a test for the presence or absence of righting reflexes after turning the animals into a supine position. It was found that in the 'bar/podium/grid' test coadministration of both NMDA receptor antagonists significantly and dose-dependently augmented morphine-induced catalepsy. The results using the AMPA receptor antagonist were less clear since the lower dose of GYKI 52466 tended to attenuate the morphine effect whereas the higher dose augmented morphine-induced catalepsy in some cases. While placing the animals on the bar and on the podium produced essentially the same results, the grid was found to be inapplicable for the measurement of morphine-induced catalepsy since the animals did not cling to the grid and fell off almost immediately after being released from the experimenter's hand. With respect to the righting reflexes it was found that the number of animals not showing these responses increased when MK-801 or CGP 37849 was coadministered with morphine. In contrast, most of the animals treated with GYKI 52466 and morphine displayed intact righting reflexes. It is concluded that glutamatergic transmission plays an important role in the mediation of morphine-induced catalepsy, though different to that of haloperidol-induced catalepsy, and that NMDA and AMPA receptors are differentially involved in different aspects of the associated behavioural state.

Keywords: Catalepsy; Catatonia; Morphine; NMDA receptor; AMPA receptor; MK-801; CGP 37849; GYKI 52466

1. Introduction

Besides many other effects, morphine has a profound influence on the behavioural activity in both humans and animals (Martin, 1984). At low doses it enhances locomotion while at higher doses it produces a biphasic effect. In the first phase, spontaneous activity is reduced, and in the second phase locomotor activity is enhanced. The strength and duration of this effect depend on the dose of morphine administered (Babbini and Davis, 1972).

At even higher doses morphine produces a state of catalepsy which is characterised by muscular rigidity and marked akinesia (lack of spontaneous activity) (Turski et al., 1982; De Ryck et al., 1980). However,

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this form of rigidity and akinesia has to be clearly distinguished from the state which occurs in Parkinson's disease brought about by degeneration of the dopaminergic afferents to the striatum and which can be modelled in animal experiments by blockade of striatal dopamine receptors, e.g. by neuroleptics such as haloperidol (Carlsson, 1993; Carlsson and Carlsson, 1990).

Other important differences between neuroleptic-induced and morphine-induced catalepsy exist. De Ryck et al. (1980) show that the former is characterised by a state of akinesia where tonic reactions subserving the maintenance of body equilibrium are excessively exaggerated and where the animals show intact righting reflexes. This exaggerated tendency towards stability is also evident from the posture the animals assume, sitting with a hunchback and limbs slightly extended sidewards. By contrast, morphine produces a rigid and akinetic state where the systems subserving body equilibrium are inactive, which is evident from the absence of postural and righting reflexes. At the same time the animals can show explosive motor behaviour, such as short bursts of vigorous running, in response to light sensory stimuli, at the end of which the animals often stop ('freeze') in the middle of a step cycle, assuming awkward positions (De Ryck et al., 1980; De Ryck and Teitelbaum, 1984; Costall et al., 1978).

Thus, while under the influence of neuroleptics static reactions are enhanced at the expense of phasic activity, under the influence of high doses of morphine the ability to display bursts of explosive behaviour, preceded and succeeded by immobility is enhanced at the expense of postural reactions. This state is viewed by some authors as a model for the 'immobility reflex' or 'animal hypnosis' displayed be some species when under a deadly threat of a predator (De Ryck and Teitelbaum, 1984). To stress the differences between these two states the morphine-induced catalepsy is sometimes referred to as 'catatonia'.

Another important difference between the two forms of catalepsy lies in the way they are influenced by *N*-methyl-D-aspartate (NMDA) receptor antagonists. It has been shown that non-competitive (Schmidt and Bubser, 1989) as well as competitive (Kretschmer et al., 1992) NMDA receptor antagonists can antagonise haloperidol-induced catalepsy, while α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists had no anticataleptic effects (Zadow and Schmidt, 1994). In contrast to this, Trujillo and Akil (1991) have shown that (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801, dizocilpine), a potent non-competitive NMDA receptor antagonist, potentiates rather than attenuates morphine-induced catalepsy. In the same experiment the combined treatment with MK-801 and morphine was lethal for many animals.

One of the few similarities of both forms of catalepsy seems to be the fact that both can be antagonised by dopamine receptor agonists (Sanberg et al., 1988; Pollard et al., 1977a).

The neurochemical and neuroanatomical substrates of the neuroleptic-induced catalepsy have received much attention and are relatively well characterised (see Schmidt et al., 1991, 1992; Carlsson, 1993; Tucci et al., 1994). In contrast to this, although there are many findings concerning morphine-induced catalepsy, no consistent model of how this behavioural state is mediated has been developped so far. Thus, after local injection into the nucleus accumbens (Costall et al., 1978; Winkler et al., 1982) and into the nucleus raphe (Broekkamp et al., 1984) morphine produces strong akinesia but no rigidity, as measured as EMG activity at the musculus gastrocnemius soleus. On the other hand, morphine injections into the striatum lead to muscular rigidity but not to akinesia (Winkler et al., 1982). It has also been shown that blocking γ-aminobutyric acid (GABA)ergic transmission in the zona incerta/lateral hypothalamus can reduce morphine-induced catalepsy (both akinesia and rigidity) (Wardas et al., 1987) while systemic administration of midazolam (Rattan and Sribanditmongkol, 1994) or atipamezole, an α_2-adrenoceptor antagonist (Weinger and Bednarczyk, 1994) augmented opiate-induced catalepsy.

It is believed that the cataleptic effects of morphine are mediated via its action on μ-opioid receptors (Havemann et al., 1980; Havemann and Kuschinsky, 1981). The precise localisation of the receptor population relevant for the described effects is unknown. However, the results of Havemann et al. (1980) suggest that the μ-opioid receptors responsible for the striatal muscular rigidity are located postsynaptically on striatal inter- and/or output-neurons.

From this it can be concluded that the different components of morphine-induced catalepsy are mediated by different anatomical and perhaps different neurochemical substrates. As mentioned above the striatum and the nucleus accumbens both seem to be critically involved in the mediation of rigidity and akinesia, respectively. Since glutamatergic transmission in these two structures is very prominent and very important for the regulation of locomotor activity (see Schmidt et al., 1991, 1992; Carlsson, 1993) it seems reasonable to assume that morphine-induced catalepsy, too, can be influenced by an interruption of glutamatergic transmission.

We set out to examine a possible role of different glutamate receptor subtypes in the mediation of morphine-induced catalepsy by combining morphine with MK-801, a non-competitive NMDA receptor antagonist, DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentoic acid (CGP 37849), a competitive NMDA receptor antagonist, and 1-(4-aminophenyl)-4-methyl-7,8-meth-

ylen-dioxy-5*H*-2,3-benzodiazepine (GYKI 52466), an AMPA receptor antagonist.

Neuroleptic-induced catalepsy is most commonly assessed by placing the animals on a bar, a podium and a grid and then measuring the time until the animals actively change their position (descent latency) (see Sanberg et al., 1988, for review). Morphine-induced muscular rigidity is often measured as the EMG activity of a limb muscle (Havemann et al., 1980; Turski et al., 1982; Wardas et al., 1987), but the bar test has also been employed (Costall et al., 1978; Bechara and Van der Kooy, 1992). Trujillo and Akil (1991) considered an

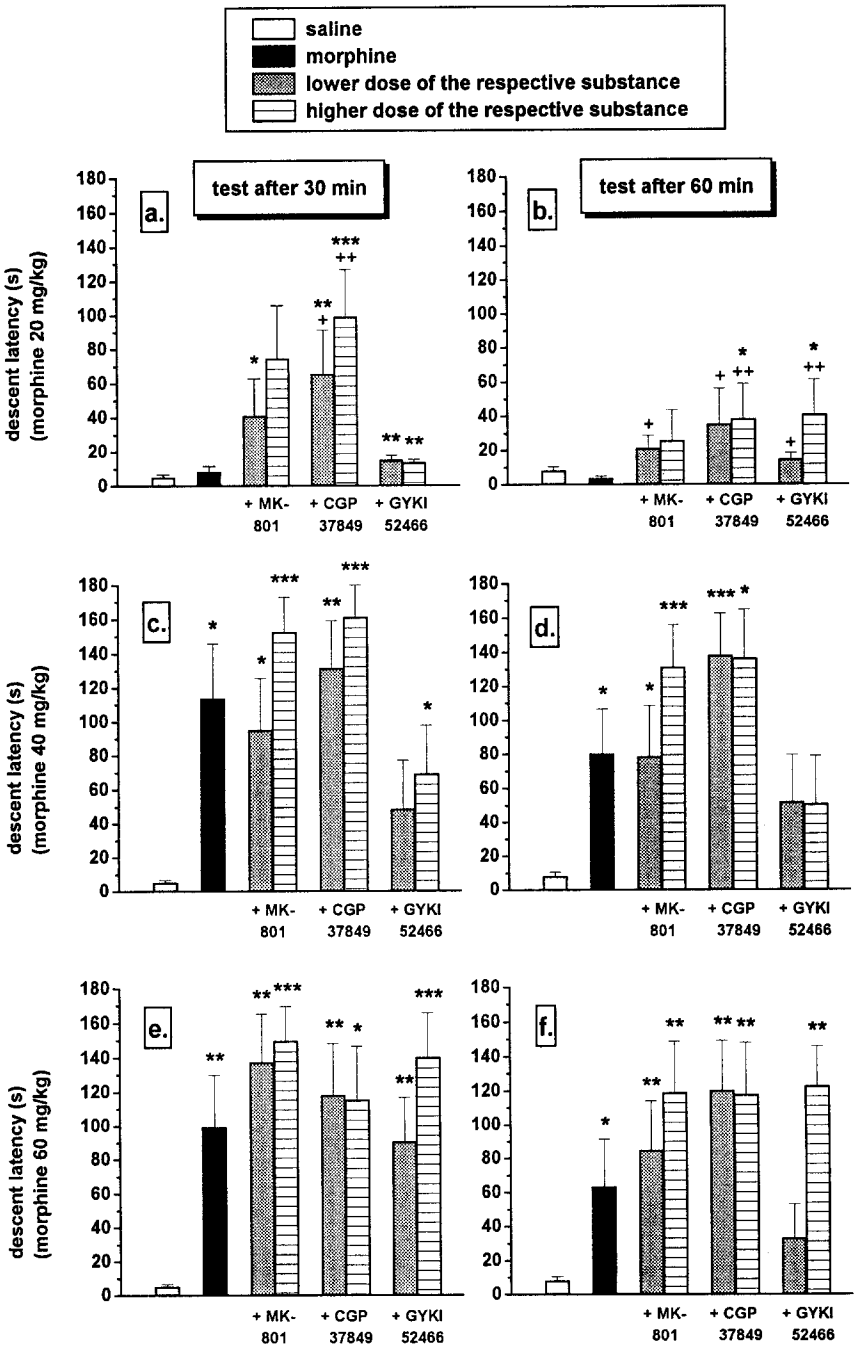

Fig. 1. Bar. Descent latency in s (mean + S.E.M., $n = 8$). (a,b) Morphine 20 mg/kg (a: test after 30 min, b: test after 60 min); (c,d) morphine 40 mg/kg (c: test after 30 min, d: test after 60 min); (e,f) morphine 60 mg/kg (e: test after 30 min, f: test after 60 min), and in combination with MK-801 (0.1 and 0.3 mg/kg), CGP 37849 (2 and 6 mg/kg) and GYKI 52466 (2 and 4 mg/kg). All injections i.p. with 1 ml/kg body weight. Kruskal-Wallis test with post-hoc Dunn's test for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$, compared to the respective morphine group.

animal as cataleptic when it did not make any attempts to right after it has been turned onto its back. We combined the last two methods in order to make a comparison of both measurements and to evaluate which of the two is better suited for measuring morphine-induced catalepsy.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Interfauna, Tuttlingen, Germany) weighing 250–300 g were used. They were

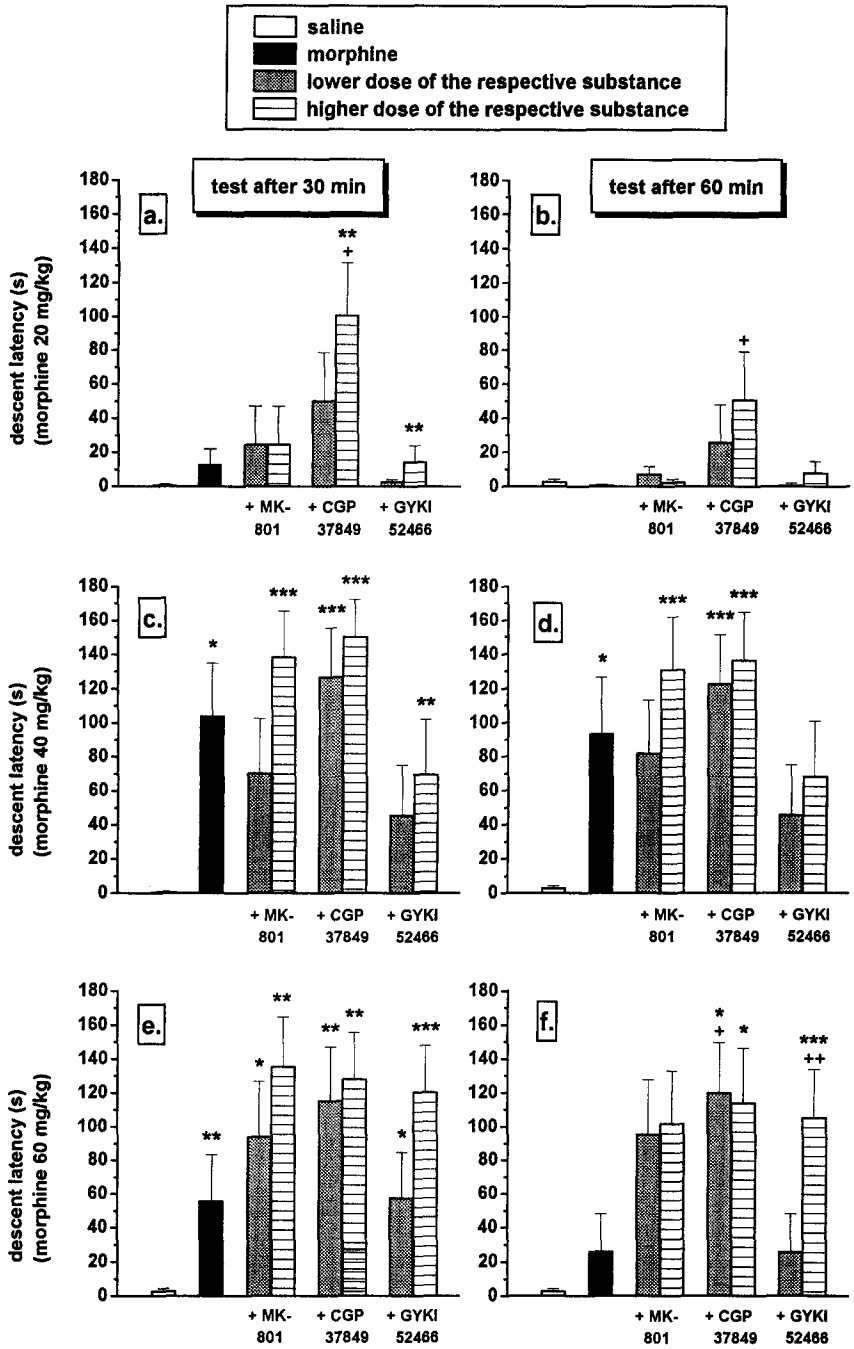

Fig. 2. Podium. Descent latency in s (mean + S.E.M., $n = 8$). (a,b) Morphine 20 mg/kg (a: test after 30 min, b: test after 60 min); (c,d) morphine 40 mg/kg (c: test after 30 min, d: test after 60 min); (e,f) morphine 60 mg/kg (e: test after 30 min, f: test after 60 min), and in combination with MK-801 (0.1 and 0.3 mg/kg), CGP 37849 (2 and 6 mg/kg) and GYKI 52466 (2 and 4 mg/kg). All injections i.p. with 1 ml/kg body weight. Kruskal-Wallis test with post-hoc Dunn's test for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the control group. $^{+}P < 0.05$, $^{++}P < 0.01$, compared to the respective morphine group.

group-housed with 6–7 rats per cage and kept under constant conditions and under a 12/12 h light cycle (lights on from 6.00 to 18.00 h). Before the start of the experiments the animals had been in our colony room for several weeks and were handled for 5 min on the 3 days prior to the experiments. Tap water was available ad libitum. Rats were fed 12 g of standard rat chow per day to reduce daily weight gain to about 1–2 g. Food was administered after the experiments when the animals had recovered from the drug effects. Experiments were carried out between 8.00 and 15.00 h.

2.2. Drugs

Morphine-sulphate (Th. Geyer, Renningen, Germany) (20, 40 and 60 mg/kg body weight) and MK-801-hydrogenmaleate (RBI, MA, USA) (0.1 and 0.3 mg/kg) were dissolved in saline. GYKI 52466 (Dr. Tarnawa, Institute for Drug Research, Budapest, Hungary) (2 and 4 mg/kg) was dissolved in distilled water. CGP 37849 (Ciba-Geigy, Basel, Switzerland) (2 and 6 mg/kg) was dissolved in saline and then adjusted to pH 7 with 1 N NaOH. All solutions were injected i.p. at 1 ml/kg body weight.

2.3. Measurement of catalepsy

Tests were carried out 30 min and 60 min after drug injection. The degree of catalepsy was determined by: (a) placing both forepaws on a horizontal bar, 9 cm above the table surface; (b) placing one forepaw on a metal podium (height 2 cm); and (c) placing the animal on a vertical grid. In all cases time was measured until the animal changed its position by displacing at least one paw. A cut-off time of 180 s was introduced after which observation was stopped if no displacement had occurred within that time. Following these tests the animals were grasped gently behind the forelimbs and slowly turned by 180° to their backs, as described by Trujillo and Akil (1991). An animal was considered cataleptic if it did not show righting reflexes or did not make other attempts to right within 15 s.

2.4. Statistics

Data from the bar/podium/grid test were analysed using the non-parametric analysis of variance (ANOVA) of Kruskal-Wallis, followed by Dunn's test for multiple comparisons. Data from the test for righting reflexes were analysed using the non-parametric Fisher's exact test (two-tailed). In both tests a $P < 0.05$ was accepted as significant.

3. Results

Neither MK-801 nor CGP 37849 nor GYKI 52466 had a significant effect in any of the tests when administered alone. In general, MK-801 tended to reduce the descent latency, and the higher dose of CGP 37849 tended to prolong the descend latency, as compared to the control group. Thus, the presentation of the results will be limited to the combined treatments with morphine.

3.1. Bar

The results for the morphine and combined treatments are depicted in Fig. 1. No significant effect was observed using 20 mg/kg morphine alone. However, a highly significant treatment effect was found after 30 min with coadministration of the glutamate receptor antagonists (Kruskal-Wallis value (KW) = 22.504, $P < 0.005$). After 60 min, comparable but weaker effects were obtained (KW = 14.076, $P < 0.05$). The levels of significance of individual treatments are given in Fig. 1a and b. With 40 mg/kg morphine, a clear prolongation of descent latencies was observed after 30 min as well as after 60 min. This effect was augmented by MK-801 and CGP 37849 and attenuated by GYKI 52466, resulting in a highly significant treatment effect at both times (KW = 23.314, $P < 0.005$ and KW = 20.811, $P < 0.005$, respectively). Individual levels of significance are given in Fig. 1c and d. 60 mg/kg morphine, too, produced a clear catalepsy with long descent latencies that were prolonged by coadministration of the NMDA receptor antagonists. The morphine effect was more pronounced after 30 min than after 60 min (treatment effect KW = 22.592, $P = 0.002$ and KW = 22.681, $P < 0.002$, respectively). The levels of significance of individual treatments are shown in Fig. 1e and f.

3.2. Podium

The results for the morphine and combined treatments are depicted in Fig. 2. No significant treatment effect across groups was observed at both times using 20 mg/kg morphine alone and in combination with the glutamate antagonists (KW = 12.787, $P > 0.05$ and KW = 9.091, $P > 0.05$, respectively). Only with the higher dose of CGP 37849 there was a clear tendency towards long descent latencies (see Fig. 2a and b). Using 40 mg/kg morphine and combined treatments, a highly significant morphine-induced catalepsy and overall treatment effect was seen after 30 min as well as after 60 min (KW = 23.532, $P < 0.002$ and KW = 21.310, $P < 0.005$, respectively). The most pronounced effects were seen when the higher dose of MK-801 and both doses of CGP 37849 were combined with morphine, while GYKI 52466 tended to reduce the morphine effect. Individual levels of significance are given in Fig. 2c and d.

Finally, with 60 mg/kg morphine alone, a clear catalepsy could be seen which was more pronounced after 30 min. However, in both tests the morphine

effect was augmented by coadministration of the glutamate receptor antagonists, resulting in a highly significant treatment effect (KW = 18.566, $P < 0.01$ and KW = 19.172, $P < 0.01$, respectively). An exception was found with the lower dose of GYKI 52466 which did not increase the morphine effect. The levels of significance of individual treatments are shown in Fig. 2e and f.

3.3. Grid

This test, which is commonly used to measure neuroleptic-induced catalepsy, was found to be completely inadequate for examining morphine-induced catalepsy, since the animals did not show any clinging reactions. They did not cling to the grid but fell off immedialety when released from the experimenter's hand. Only

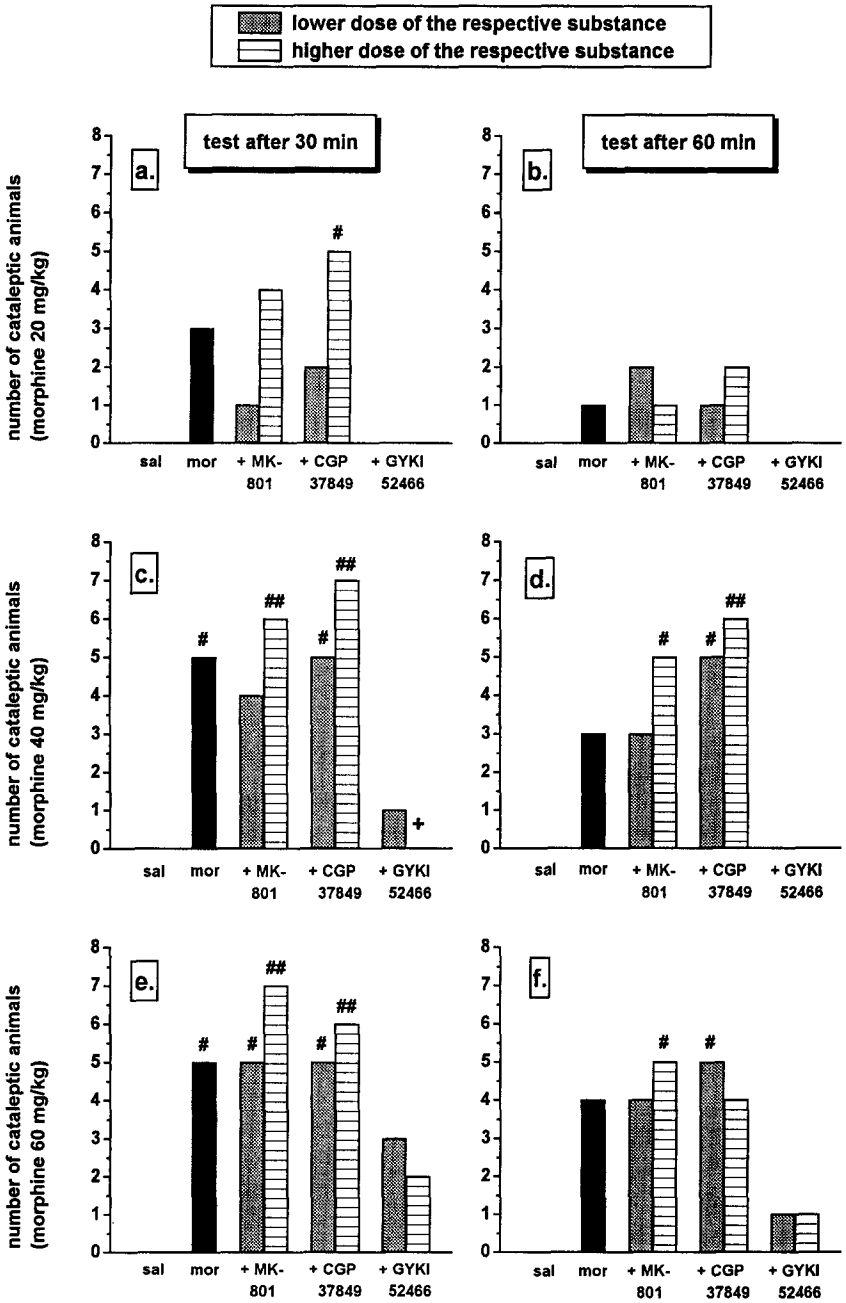

Fig. 3. Test for righting reflexes. Number of animals per treatment group rated cataleptic. (a,b) Morphine 20 mg/kg (a: test after 30 min, b: test after 60 min); (c,d) morphine 40 mg/kg (c: test after 30 min, d: test after 60 min); (e,f) morphine 60 mg/kg (e: test after 30 min, f: test after 60 min), and in combination with MK-801 (0.1 and 0.3 mg/kg), CGP 37849 (2 and 6 mg/kg) and GYKI 52466 (2 and 4 mg/kg). All injections i.p. with 1 ml/kg body weight. Statistical analysis was done with Fisher's exact test (two-tailed). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to the control group. $^{+}P < 0.05$, compared to the respective morphine group.

animals treated with morphine and GYKI 52466 showed clinging reactions to some extent. This resulted in similar (short) descent latencies as in control animals. No significant treatment effect was seen with any dose of morphine alone and in combination with the glutamate receptor antagonists (data not shown).

3.4. Test for righting reflexes

The results of this test are shown in Fig. 3. While the higher doses of MK-801 and CGP 37849, administered together with morphine, generally increased the number of animals that were rated cataleptic, GYKI 52466 in both doses clearly decreased the number of animals showing no righting reflexes. Almost all of the animals receiving the AMPA receptor antagonist in addition to morphine showed intact postural reactions.

3.5. Further observations

In general, the animals responded very differently to morphine and combined treatments with MK-801 and CGP 37849, almost like in an 'all-or-nothing' manner, i.e. the animals were either strongly cataleptic (descent latencies of > 100 s) or they behaved almost like control animals (descent latencies of a few seconds). This considerable variation is also evident from the large standard errors and lack of statistically significant group differences despite clear differences in the group means. Using a combination of morphine and GYKI 52466, the animals' responses were much more uniform, resulting in small standard errors and significant differences to the control group, even when the differences of the means were considerably smaller than with the NMDA receptor antagonists (see e.g. Fig. 1a).

Three animals died during the course of the experiment within the 12 h following drug injection. Two of them had received 40 mg/kg morphine plus 0.3 mg/kg MK-801 and one had received 60 mg/kg morphine plus 0.3 mg/kg MK-801. All other animals survived their respective treatment even though the combination of morphine and the NMDA receptor antagonists obviously caused considerable respiratory depression in many animals.

Many animals, irrespective of their treatment, showed hoarding behaviour, i.e. collecting and carrying around cage bedding.

4. Discussion

A dose of 20 mg/kg morphine alone was not sufficient to produce a clear cataleptic state, while at the higher doses a significant catalepsy was observed. This effect tended to be more pronounced after 30 min than after 60 min, which is consistent with the behavioural profile after higher morphine doses (Babbini and Davis, 1972). The effects were essentially the same for both bar and podium, and for both tests 30 min and 60 min after drug injection, so these results will be discussed together below.

In most cases morphine-induced catalepsy was markedly augmented by the non-competitive NMDA receptor antagonist MK-801. This is in line with the results of Trujillo and Akil (1991). In addition, it was shown for the first time that the competitive NMDA receptor antagonist CGP 37849 also potentiates the cataleptic effect of morphine. In some cases this interaction seemed to be even stronger than in the case of MK-801. A quite different picture was observed following coadministration of the AMPA receptor antagonist GYKI 52466. When combined with 20 mg/kg morphine, the descent latency was prolonged, but this effect was not as strong as with the NMDA receptor antagonists. When combined with 40 mg/kg morphine, the descent latency tended to be reduced, indicating that GYKI 52466 partially antagonised the cataleptic effect of morphine. Finally, in combination with 60 mg/kg morphine the lower dose of GYKI 52466 reduced while the higher dose prolonged descent latencies at the bar and the podium.

The grid proved to be completely inadequate to measure morphine-induced catalepsy, since the animals did not cling to the grid but fell off immediately when released from the experimenter's hand. Only animals treated with morphine and GYKI 52466 showed clinging reactions to some extent. This resulted in similar (short) descent latencies as in control animals.

Quite similar results as in the bar/podium test were obtained with the subsequent test for presence or absence of righting reflexes. Again, both NMDA receptor antagonists augmented morphine-induced catalepsy which was evident from an increased number of animals showing no attempts to right. The anticataleptic effects of GYKI 52466 were more evident in this test. Both doses clearly reduced the number of animals with absent righting reflexes.

Thus, the comparison of both tests shows that they yielded qualitatively quite similar results. The bar and the podium tests are well suited to measure the akinetic component of morphine-induced catalepsy. Since the absence of posture reflex reactions is a very characteristic feature of that state, combination of the bar/podium test and reflex test appears to be a good method to measure the cataleptic effects of morphine and the influence of other substances thereupon. However, neither of the tests allows direct evaluation of the second component of morphine-induced catalepsy, muscular rigidity. In order to characterise the effects of morphine in combination with other substances more comprehensively, one would have to combine the above-mentioned tests with additional electromyo-

graphic recordings. This would allow to determine possible differential effects, e.g. of NMDA receptor antagonists on akinesia on the one hand and rigidity on the other hand.

As mentioned above, our results are in good agreement with the results of Trujillo and Akil (1991). In both experiments morphine-induced catalepsy was augmented by MK-801. However, the very high mortality under the combined treatment found by these authors was not observed in our study. These authors found that a combination of 30 mg/kg morphine with 0.3 mg/kg MK-801 was lethal for 100% of the animals receiving this treatment. In our study, although the combination of morphine and the NMDA antagonists obviously caused considerable respiratory depression in many animals, three animals died. Two of them had received 40 mg/kg and one 60 mg/kg morphine, combined with 0.3 mg/kg MK-801 in each case. The reason for the high mortality in the above-mentioned study is unclear, especially since rats of the same strain (Sprague-Dawley) were used. Other studies also failed to observe such a high lethality of morphine plus MK-801 (Jeziorski et al., 1994).

In this study it was shown for the first time that a competitive NMDA receptor antagonist (CGP 37849) can also potentiate morphine-induced catalepsy. Thus, as in the case of MK-801, there is a clear discrepancy in the way CGP 37849 affects haloperidol- and morphine-induced catalepsy. The former is antagonised by CGP 37849 (Kretschmer et al., 1992) while the latter is augmented (present results). A differential effect in both forms of catalepsy was also found for GYKI 52466. This substance does not counteract a haloperidol-induced catalepsy, even at higher doses than those used in the present study (Zadow and Schmidt, 1994) and even attenuates the anticataleptic effect of MK-801 (Hauber and Andersen, 1993) in the case of neuroleptic-induced catalepsy. In contrast to this, GYKI 52466 was, with some exceptions, able to at least partly antagonise the morphine-induced catalepsy, an effect which was most evident with respect to the posture reflexes.

These results suggest that there are important and substantial differences in the way haloperidol- and morphine-induced catalepsy are mediated. The former is produced by a blockade of dopamine receptors on GABAergic efferents of the striatum or, in the case of Parkinson's disease, by a lack of dopaminergic input from the substantia nigra pars compacta to the striatum (Carlsson and Carlsson, 1990; Jolicoeur and Rivest, 1992). Nigral dopamine and cortical glutamate in the striatum seem to have opposite effects on the activity of striatal projection neurons and on behavioural output (Kötter, 1994; Carlsson and Carlsson, 1990). Thus, if dopaminergic transmission is reduced in the striatum either by dopamine receptor blockade or by dopamine denervation, the glutamatergic tone in the striatum prevails, leading to catalepsy. A blockade of NMDA receptors by MK-801 or CGP 37849 would reduce the glutamatergic tone, thus restoring the initial balance of dopamine and glutamate in the striatum. This scenario would explain the anticataleptic effects of NMDA receptor antagonists in the case of haloperidol-induced catalepsy (Schmidt et al., 1992; Carlsson, 1993).

Since there is no good explanation for the mediation of morphine-induced catalepsy so far, it is difficult to interpret the results of this study. This form of catalepsy does not seem to be exclusively mediated by the nigrostriatal system and not exlusively by dopaminergic mechanisms (see Introduction). The cataleptic effect of morphine could be explained by presynaptic μ-opioid receptors located on dopaminergic axon terminals in the striatum or nucleus accumbens, which would block dopamine release, thus leading to catalepsy. Indeed, presynaptic opioid receptors have been postulated based on the finding that [^{3}H]Leu-enkephalin binding in the striatum and [^{3}H]naloxone binding in the nucleus accumbens were significantly reduced following destruction of the nigrostriatal projection or electrocoagulation of A10 dopaminergic neurons, respectively (Pollard et al., 1977a, b). However, this scenario is highly unlikely for several reasons. First, it was shown that dopamine release in the striatum was not significantly altered following morphine treatment (Havemann et al., 1980). Second, it has been demonstrated that the presynaptic opioid receptors are very probably δ- and not μ-type receptors, and that the μ-opioid receptors in the striatum are located postsynaptically (Petit et al., 1986; Trovero et al., 1990). Finally, this model would not explain the results of the present study, since it would predict that, for the above-mentioned reasons, NMDA receptor antagonists would attenuate morphine-induced catalepsy instead of augmenting it as was found in this study.

Thus, one has to assume that the cataleptic effect of morphine is not mediated by an impairment of dopaminergic transmission in the striatum. This, however, does not exclude the possibility that there is an interaction of morphine with dopaminergic transmission in other brain areas, especially since it has been shown that morphine-induced catalepsy can be antagonised by dopamine agonists (Sanberg et al., 1988; Pollard et al., 1977a).

If the cataleptic effect of morphine was mediated by μ-opioid receptors located postsynaptically on striatal output neurons, one would have to postulate that these receptors are only or at least preferentially located on neurons belonging to the 'direct' pathway, projecting from the striatum via the substantia nigra pars reticulata/globus pallidus pars interna to thalamic nuclei, but not on neurons belonging to the 'indirect' pathway, projecting from the striatum via the globus pallidus pars externa, subthalamic nucleus and substantia nigra

pars reticulata/globus pallidus pars interna to thalamic nuclei (Carlsson and Carlsson, 1990; Schmidt et al., 1992). Only if this was the case, activation of these μ-opioid receptors could lead to a decrease of activity in thalamic motor nuclei and, as a result of this, to reduced motor activity and symptoms of catalepsy.

Independently of this line of reasoning, a possible role of the serotonergic system (Broekkamp et al., 1984), the adrenergic system (Weinger and Bednarczyk, 1994; Weinger et al., 1995) the GABAergic system (Wardas et al., 1987) and benzodiazepine receptors (Rattan and Sribanditmongkol, 1994) in the mediation of morphine-induced catalepsy is discussed as well. A possible role of excitatory amino acids in the striatum was pointed out by Turski et al. (1982). Based on their findings, they concluded that in the striatum morphine might act as a functional antagonist of glutamate, either by blocking glutamate release through activation of presynaptic μ-opioid receptors or by an antagonistic interaction with glutamate postsynaptically on striatal output neurons. This antagonism is thought to be important for the emergence of cataleptic symptoms, mainly rigidity. These assumptions could indeed explain why NMDA receptor antagonists do potentiate morphine-induced catalepsy. If morphine antagonises glutamatergic transmission, leading to catalepsy, then NMDA receptor antagonists which also reduce glutamatergic transmission, would act in the same direction as morphine, thus potentiating morphine's effect. However, this model has to be rejected for several reasons. First, it would not explain the rather anticataleptic effects of GYKI 52466, which also reduces glutamatergic transmission. Second, it has been shown that intrastriatal injections of non-competitive NMDA receptor antagonists produce locomotor stimulation rather than akinesia and rigidity, as would be predicted by the model (Bubser et al., 1992; Schmidt et al., 1992). Also, local injections of competitive NMDA receptor antagonists into the striatum do not produce catalepsy (Schmidt et al., 1992; Bubser et al., 1992). Finally, it has been shown that local application of the receptor agonist NMDA into the rostral striatum depresses motor behaviour (Schmidt and Bury, 1988), augments haloperidol-induced catalepsy (Mehta and Ticku, 1990) and produces muscular rigidity (Klockgether and Turski, 1993), i.e. it acts like morphine and not as its antagonist.

Thus, although the above-mentioned findings argue for an important role of the striatum in the mediation of morphine-induced rigidity, the basic mechanisms and the location of the relevant μ-opioid receptor population are largely unknown. Even less is known about the action and location of those μ-opioid receptors that mediate the akinetic component of morphine-induced catalepsy. There is evidence that the nucleus accumbens may play an important role (Costall et al., 1978; Winkler et al., 1982). Interesting in this context, but difficult to reconcile with our results, is the observation that local injection of the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) into the nucleus accumbens in addition to a systemic injection of 10 mg/kg morphine, produces a marked muscular rigidity (Layer et al., 1993).

The results of recent studies examining other brain structures are in part contradictory. Thus, in one study it was shown that lesions of the pedunculopontine nucleus can completely block morphine-induced catalepsy (Olmstead and Franklin, 1994), whereas in another study no such effect of a lesion of this nucleus has been found (Bechara and Van der Kooy, 1992).

In conclusion, we examined the influence of a competitive and a non-competitive NMDA receptor antagonist and of an AMPA receptor antagonist on morphine-induced catalepsy. It was found that this form of catalepsy (in contrast to neuroleptic-induced catalepsy) was markedly augmented by the NMDA receptor antagonists but was partly attenuated by the AMPA receptor antagonist in most cases. The morphine-induced catalepsy is characterised by an almost complete absence of righting and posture reflexes, which is again in contrast to the neuroleptic-induced state. Again, this effect was augmented by the NMDA receptor antagonists and antagonised by the AMPA receptor blocker.

It is concluded that morphine-induced and neuroleptic-induced catalepsy are mediated by markedly different mechanisms, and both forms should be clearly discriminated. Whatever the precise neuronal substrate may be, from the results of this study it is clear that glutamatergic mechanisms play a very important role in the mediation of morphine-induced catalepsy and that NMDA and AMPA receptors are differentially involved in the different symptoms characterising this state. While the NMDA receptors seem to be critically involved in the mediation of rigidity and akinesia, the AMPA receptors seem to play a more important role in the coordination and execution of reflexive behaviours subserving the maintenance of body equilibrium.

Finally, the view of Trujillo and Akil (1991) is supported that greatest care should be taken when considering coadministration of morphine and NMDA receptor antagonists for whatever therapeutical reasons in humans.

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