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Phenytoin Reversal and Prevention of Morphine-Induced Catalepsy in the Rat[1,2]

SUSAN L. COOKSON AND J. DOUGLAS MANN

Department of Neurology, University of North Carolina, Chapel Hill, NC 27514

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COOKSON, S. L. AND J. D. MANN. *Phenytoin reversal and prevention of morphine-induced catalepsy in the rat.* PHARMAC. BIOCHEM. BEHAV. 12(5) 743–746, 1980.—The interaction of morphine sulfate and phenytoin was studied with respect to morphine-induced catalepsy in the rat using a previously described method for measuring degree of immobility. As expected, catalepsy developed several minutes after intravenous morphine and lasted for more than ninety minutes. Intravenous phenytoin, 35 mg/kg, given fifteen min prior to or following the administration of morphine, 1 mg/kg, significantly prevented, or reversed, morphine-induced catalepsy. Control experiments revealed that the antagonistic effect was due solely to the morphine-phenytoin interaction. A similar effect was found with naloxone, 0.2 mg/kg. The data are discussed in terms of possible sites of interaction, including synaptosomal calcium metabolism.

Phenytoin Morphine Naloxone Catalepsy Morphine-induced catalepsy Rat Bar test Phenytoin-morphine antagonism

ADMINISTRATION of morphine to naive rats results in a prolonged state of waxy rigidity and immobility termed catalepsy. With intraperitoneal injections, morphine-induced catalepsy in rats has been variously reported to reach a maximum within 10 to 40 min [8,12], while catalepsy appears within four min in rats given intravenous morphine and is sustained for more than 90 min. It has been proposed that increased turnover of dopamine is related to morphine-induced catalepsy. Homovanillic acid (HVA), a metabolite of dopamine, is significantly increased in the striatum following acute morphine administration in the rat. However, peak HVA levels are achieved 90 to 150 min after morphine is given [12], at a time when morphine-induced catalepsy is declining. Additionally, ablation of the striatum does not prevent the appearance of catalepsy when morphine is given [11], suggesting that neurotransmitters other than, or in addition to, dopamine are important in the development of catalepsy.

Coincident with the appearance of catalepsy, there is a significant decrease in calcium concentration in all brain regions within 10 min of morphine administration. Maximum depletion of brain calcium occurs 30 min after intraperitoneal morphine, except in hypothalamus and hippocampus where depletion is maximum at 60 min [1]. Reduction in calcium concentration is found only in the synaptosomal fraction [22].

The importance of calcium depletion in the development of catalepsy is also supported by studies showing that naloxone both prevents and rapidly reverses morphine-induced catalepsy [21] as well as the associated depletion of synaptosomal calcium [18].

In the present study, the pharmacologic interaction of morphine and phenytoin was studied to further test the hypothesis that synaptosomal calcium metabolism is critical to the development of catalepsy. Phenytoin was chosen as an agent which alters membrane calcium flux and which has minimal effects on the dopamine neurotransmitter system. Behavioral responses were studied in the rat with respect to the effects of phenytoin given prior to or following a single intravenous dose of morphine. An antagonism between morphine and phenytoin was clearly demonstrated, in that there was both prevention and reversal of morphine-induced catalepsy by phenytoin.

METHOD

Sixty adult naive Sprague Dawley rats of the Charles River Strain, weighing between 250 and 400 g, were used for all experiments. Under ether anesthesia, an indwelling venous catheter (Clay Adams, PE 20) was placed in the right external jugular vein and externalized at the dorsal midline near the base of the neck. A recovery time of two days was allowed prior to experimentation, during which time animals were maintained in a constant temperature environment with a 12 hour on-off light cycle and supplied with Purina rat chow and water ad lib.

Responses to intravenous injections were assessed using a modification of the bar test previously described for quantitating motor immobility in rats [12]. Animals were positioned with forepaws on a stationary bar 7.5 cm high, and time spent on the bar was measured. Forty-five seconds was used as the maximum time for each trial. Six trials were conducted for each time point and the testing procedure was

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[2]Reprint requests should be sent to: Dr. J. Douglas Mann, Department of Neurology, 751 Burnett-Womack Building Clinical Sciences Building 229H, University of North Carolina, Chapel Hill, NC 27514.

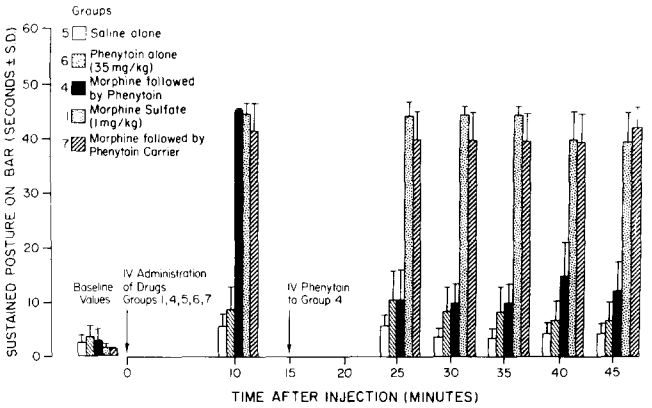

FIG. 1. Quantitative comparison of the duration of sustained motor immobility in control and experimental groups. Phenytoin given after morphine (Group 4) results in rapid reversal of the catalepsy and sustained normalization of motor behavior.

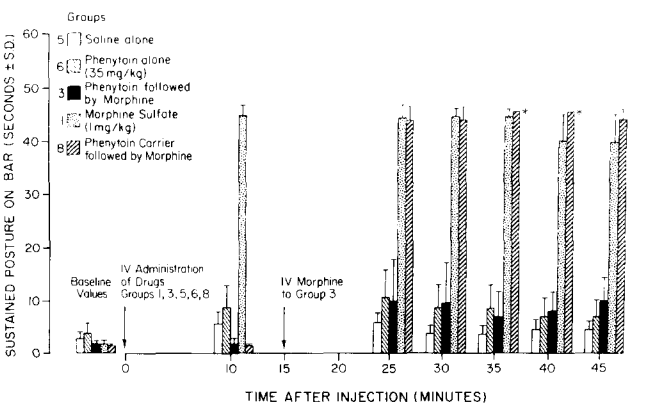

FIG. 2. Comparative effects of pre-treatment with phenytoin plus carrier and phenytoin carrier alone on the appearance of catalepsy when morphine is given compared to control groups. *Time sustained on bar longer than 45 second cut off period, therefore standard deviation equals zero.

performed every five minutes for one hour after the initial injections. External environmental stimuli including light, sound and temperature were standardized, and animals were tested between 9 a.m. and 1 p.m.

Ten groups of animals, each consisting of six naive rats, were tested before and after injection of the following pharmacologic agents: Group 1—morphine sulfate (Mallincrodt) in saline; Group 2—phenytoin carrier—20% propylene glycol and 5% ethanol; Group 3—phenytoin (Parke Davis) followed 15 min later by morphine sulfate (all phenytoin was given in carrier solution described above); Group 4—morphine sulfate followed 15 min later by phenytoin; Group 5—saline; Group 6—phenytoin; Group 7—morphine followed 15 min later by phenytoin carrier; Group 8—phenytoin carrier followed 15 min later by morphine; Group 9—morphine followed five minutes later by naloxone (Endo Laboratories); and Group 10—naloxone followed five minutes later by morphine. Morphine treated animals received a dose of 1 mg/kg of morphine sulfate in normal saline. The dose of naloxone was 0.2 mg/kg in saline. The dose of phenytoin was 35 mg/kg, with the concentration of phenytoin in carrier being 50 mg/cc. When carrier was used without phenytoin, the volume given was calculated according to the amount needed to deliver phenytoin at a dose of 35 mg/kg. Serum phenytoin levels were determined by gas chromatography [6] 60 min after phenytoin was given.

RESULTS

Intravenous morphine sulfate, 1 mg/kg, (Group 1) consistently produced the characteristic cataleptic response of prolonged motor immobility, waxy rigidity and stiffness of the tail and body with no apparent decrease in level of consciousness. Catalepsy continued for at least 60 min after morphine administration, with time spent in a fixed position on the bar averaging 39.7 ± 10.0 (SD) sec (Fig. 1). Injection of saline alone (Group 5), as expected, produced no body rigidity or immobility. Time spent on the bar for that group was 5.5 ± 4.3 sec. No cataleptic behavior was observed when phenytoin, 35 mg/kg, (Group 6) was given alone. Phenytoin produced mild incoordination of the hind legs in some animals which resolved completely within fifteen minutes in all instances. Serum phenytoin levels one hour after administration were 24.0 ± 1.7 μg/ml.

We first attempted to determine if morphine-induced catalepsy, once established could be reversed by phenytoin. Catalepsy appeared within four minutes of intravenous morphine, and prior to the injection of phenytoin in morphine treated animals, mean time on the bar was 44.9 ± 0.4 sec. As shown in Fig. 1, there was a dramatic reversal of the cataleptic response with relaxation of body stiffness and normalization of motor activity within 10 min of intravenous phenytoin. Reversal was sustained for at least 30 min. Animals returned to their pre-morphine activity level showing no apparent residual effects.

Giving phenytoin before morphine in the same doses (Group 3) effectively prevented the appearance of catalepsy as shown in Fig. 2. Sustained immobility increased slightly in the phenytoin pretreatment group after morphine was given, but there was no significant difference between that group and saline or phenytoin controls (Groups 5 and 6). Body and tail stiffness were not observed and animals exhibited normal activity throughout the period of observation.

Phenytoin carrier, 20% propylene glycol and 5% ethanol, was tested for its effects on morphine-induced behavioral changes. Figure 1 illustrates that carrier alone, administered after catalepsy was well established (Group 7), had no significant effect in reversing the response, in marked contrast to Group 4 where phenytoin and its carrier were both given after morphine. The failure of carrier alone to prevent catalepsy (Group 8) is shown in Fig. 2, and is contrasted with Group 3 where phenytoin and carrier were given prior to morphine. Injection of phenytoin carrier alone (Group 2) produced results similar to Group 6, phenytoin plus carrier; time on the bar was 10.4 ± 10.2 and 9.3 ± 13.3 seconds respectively. Groups 2 and 6 were not significantly different from saline treated animals with respect to time spent on the bar.

As expected, naloxone both rapidly and consistently prevented and reversed the development of morphine catalepsy. Reversal was evident within 2 min of IV naloxone, and the effect was persistent for 30 to 50 min (Group 9). Naloxone also prevented the development of catalepsy when given in advance of morphine (Group 10). (Results not shown in Figs. 1 or 2).

DISCUSSION

Morphine, chlorpromazine [4], and haloperidol [12] have

all been reported to induce a state of catalepsy. However, catalepsy is not a unitary phenomenon and the origin of this behavioral response from the standpoint of neuroanatomical regions and neurotransmitter systems is apparently different for each of these agents. For example, bilateral ablation of the corpus striatum markedly attenuates chlorpromazine-induced catalepsy while enhancing the cataleptic response to morphine [11]. Additionally, morphine-induced catalepsy is not reversed by anticholinergic agents such as atropine, benztropine and scopolamine, in contrast to the cataleptic response to haloperidol, which is significantly reduced by those drugs [7].

Nearly all major neurotransmitter systems are effected by acute morphine administration. Morphine increases the turnover of striatal and mesolimbic dopamine [3]. In rat hypothalamus, cerebellum, brainstem, and other non-striatal brain regions, single dose morphine significantly increases the major metabolite of norepinephrine [16]. Acute administration of morphine also increases brain serotonin turnover in the rat [23]. Acetylcholine turnover is decreased in the limbic system and unchanged in the striatum with exposure to morphine in one strain of mice, while the opposite is found in another strain [15]. Hence it is likely that catalepsy in response to morphine is the net result of activation of multiple neurotransmitter systems in many brain regions, thus lacking some of the specificity of catalepsy induced by dopaminergic blocking agents such as haloperidol. Furthermore, a lack of neuroanatomical specificity is suggested by the finding that morphine catalepsy is reversed when naloxone is injected into either the caudate nucleus or periaquaductal gray matter [21].

It is likely that activation of neurotransmitter systems by morphine is related to its effects on membrane calcium metabolism. Single dose morphine in naive rats results in a decrease in calcium in all brain regions [1]. Decreased calcium concentration occurs in the synaptosomal particulate fractions of rat brain [22], with no change in sodium, potassium, or magnesium concentrations [18].

It has been suggested that decreased synaptosomal calcium following morphine represents a dissociation of calcium from synaptic membrane. Calcium translocation at the synapse could enhance membrane ionic permeability and excitability through increased adenylate cyclase activity, generation of cyclic-AMP (c-AMP), and phosphorylation of neural membrane proteins [13,20]. Other studies have revealed that morphine increases both adenylate cyclase activity [10,14] and c-AMP [19], as well as inhibiting phosphodiesterase [14], the major enzyme which breaks down c-AMP.

In terms of the above mechanisms for enhancement of membrane excitability, phenytoin would be expected to counteract morphine due to the inhibitory effect it has on calcium dependent phosphorylation of membrane protein [5]. Additionally, phenytoin has been shown to inhibit c-AMP formation under conditions where there is pharmacologic stimulation of c-AMP production [9]. Alternatively, if the apparent morphine depletion of synaptosomal calcium represents an actual loss of calcium from the region of the synapse rather than a change in membrane binding, phenytoin would counteract the effects of morphine due to its properties of inhibiting calcium flux [9] and stabilizing neural membrane in a low calcium environment [2,17]

Finally, the hypothesis that catalepsy depends specifically on changes in synaptosomal calcium metabolism is supported both by our findings that phenytoin and naloxone prevent and reverse morphine catalepsy, and the earlier demonstration that naloxone both prevents and reverses the synaptosomal calcium depletion associated with administration of morphine [18].

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