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EFFECT OF MORPHINE AND NALOXONE ON PRIMING-INDUCED AUDIOGENIC SEIZURES IN BALB/c MICE

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1 Morphine (1–200 mg/kg, s.c.) reduced the incidence and prolonged the latency of priming-induced audiogenic seizures in a dose-dependent manner.

2 This effect was reversed by naloxone (1 and 2 mg/kg) although naloxone was itself inactive.

3 This priming-induced seizure model may be useful in the study of tolerance and physical dependence.

Introduction

Many drugs have been shown to suppress the incidence and severity of audiogenic seizures (that is, seizures induced by a loud sound). These seizures can be suppressed by centrally acting drugs such as tranquillizers (Plotnikoff, 1960) and sedatives (Collins & Horlington, 1969). However, little has been done to investigate the effects of morphine and its antagonism by naloxone in this test. Morphine has been investigated by Collins & Horlington (1969) and Boggan (1973) and apomorphine has been shown to suppress audiogenic seizures (Anlezark & Meldrum, 1975).

Seizure-resistant strains of mice can be made reliably and consistently seizure-prone, by comparison with spontaneously seizure-prone mice, by priming procedures (SJL/J mice: Fuller & Collins, 1968; CF-1 mice: Iturrian & Fink, 1968; BALB/c mice: Henry & Bowman, 1969; heterogeneous strain: Boggan, Freedman & Lovell, 1971; Gates & Chen, 1976) and thus the priming procedure (as described below) may facilitate pharmacological studies (Iturrian & Johnson, 1970).

The aim of this study was to test morphine and its antagonist naloxone using priming-induced audiogenic seizures.

Methods

Twenty-one day old (±1 day) BALB/c mice were primed by 25 s exposure to a bell of intensity 131 dB re 0.0002 dyn cm^{-2} and tested for seizures 9 days (±1 day) later by re-exposure to the same acoustic stimulus for a maximum of 60 s or until seizure occurred. The parameters for priming in this line of mice have been described elsewhere (Chen; 1973). The incidence of four stages of audiogenic seizures was recorded (wild running, clonic seizure, tonic seizure and death) as were the latencies of onset of the first two stages.

Control injections and drugs were administered on a split litter basis. In the first experiment the effects of saline, naloxone and morphine were tested alone. Saline (0.9% w/v NaCl solution) or morphine was given 20 min before testing and 15 mice were used at each dose level (1, 2.5, 7.5, 25, 50, 100 and 200 mg/kg) of morphine. Naloxone was given to 20 mice at each of four doses (1, 5, 10 and 20 mg/kg), 20 min before testing. In the second experiment the effect of the combination of morphine and naloxone given 20 min before testing was studied. Four doses of morphine (7.5, 50, 200, 400 mg/kg) were tested alone and in combination with 1 and 2 mg/kg of naloxone. Each group contained 20 mice.

Drugs

Morphine hydrochloride (McFarlane Smith) and naloxone hydrochloride (Bristol Laboratories) were used. All drugs were given subcutaneously in a volume of 0.1 ml per 10 g weight.

Results

As saline-injected mice showed no significant difference from those not injected, in each of the

morphine and naloxone experiments these groups were pooled to form control groups with which drug effects were compared.

In the first experiment naloxone was found to be ineffective in altering the incidence of any audiogenic seizure component (Fisher Exact test, $P > 0.1$ in each case, two tailed, Table 1) or in altering the latencies of wild running (Kruskal-Wallis, $H(3) = 1.605$, $P > 0.5$) or of clonic seizure (Kruskal-Wallis, $H(3) = 6.539$, $P > 0.05$). However, morphine reduced the incidence of all seizure components and prolonged the latency of wild running (Kruskal-Wallis $H(6) = 24.618$, $P < 0.001$) and clonic seizure (Kruskal-Wallis, $H(6) = 27.484$, $P < 0.001$). The suppression by morphine was dose-dependent and the lowest dose which significantly reduced clonic seizures was 1 mg/kg (Fisher Exact test, $P < 0.05$, two tailed). The lowest dose which significantly reduced wild running, clonic and tonic seizures was 2.5 mg/kg (Fisher Exact test, $P < 0.005$, two tailed) while all higher doses significantly reduced wild running and clonic seizures and completely inhibited the tonic seizure stage (Table 1). The initially low incidence of death (20%) was not significantly altered by morphine although doses of 7.5 mg/kg and above completely abolished it. However, it was impossible to abolish completely either wild running or clonic seizure, and the effect of morphine reached a maximum at 50 mg/kg, when these seizure stages were reduced from 100% to 33.3% and 26.7% respectively.

The results of the second experiment as shown in Figure 1 indicate the antagonistic effect of naloxone on the suppression of audiogenic seizures by morphine. At all doses of morphine, 2 mg/kg of naloxone produced a significant reversal of suppression of the first three stages of audiogenic seizures to a level not significantly different from that in mice treated with naloxone alone.

Naloxone, at a dose of 1 mg/kg, significantly antagonized the suppressive effects of 7.5, 50 and 400 mg/kg of morphine on wild running, clonic and tonic seizure (see Figure 1, Fisher Exact test, $P < 0.05$ in each case). However, naloxone, at this dose, did not significantly antagonize the effect of 200 mg/kg of morphine. This result is difficult to interpret but may be related to the well known biphasic excitant-depressant action of morphine (Tatum, Seevers & Collins, 1929) and the ability of morphine to cause convulsions in some species at high doses (Hazelton & Koppanyi, 1941). Although the low incidence of death in these animals prevented the use of this factor in analysis, 1 mg/kg of naloxone did produce a significant reversal of the protective action of 7.5 mg/kg of morphine (Fisher Exact test, $P < 0.01$, two tailed). Similarly 2 mg/kg of naloxone significantly reversed the effect of 50 mg/kg of morphine on death (Fisher Exact test, $P < 0.05$, two tailed).

Analysis of the latencies of wild running and clonic seizures indicates that, irrespective of the presence of naloxone, morphine causes a dose-dependent significant increase in the latency of these parameters (Kruskal-Wallis, W.R., $H(3) = 24.496$, $P < 0.001$; clonic seizure, $H(3) = 26.176$, $P < 0.001$). Naloxone

Table 1 Effect of naloxone and morphine on the incidence of audiogenic seizures in BALB/c mice

Treatment (doses in mg/kg)	n	*Incidence of seizure component* W	C	T	D
Control	40	39	33	20	9
Naloxone					
1	20	17	15	11	8
5	20	17	13	10	8
10	20	20	18	9	7
20	20	19	13	7	5
Control	40	40	40	28	8
Morphine					
1	15	12	11*	8	5
2.5	15	11*	11*	2*	1
7.5	15	11*	8*	0*	0
25.0	15	6*	4*	0*	0
50.0	15	5*	4*	0*	0
100.0	15	7*	6*	0*	0
200.0	15	8*	6*	0*	0

W: wild running; C: clonic seizure; T: tonic seizure; D: death.
Asterisks indicate which groups, when compared with controls using a two-tailed Fisher Exact test, were significantly different at the 5% probability level.

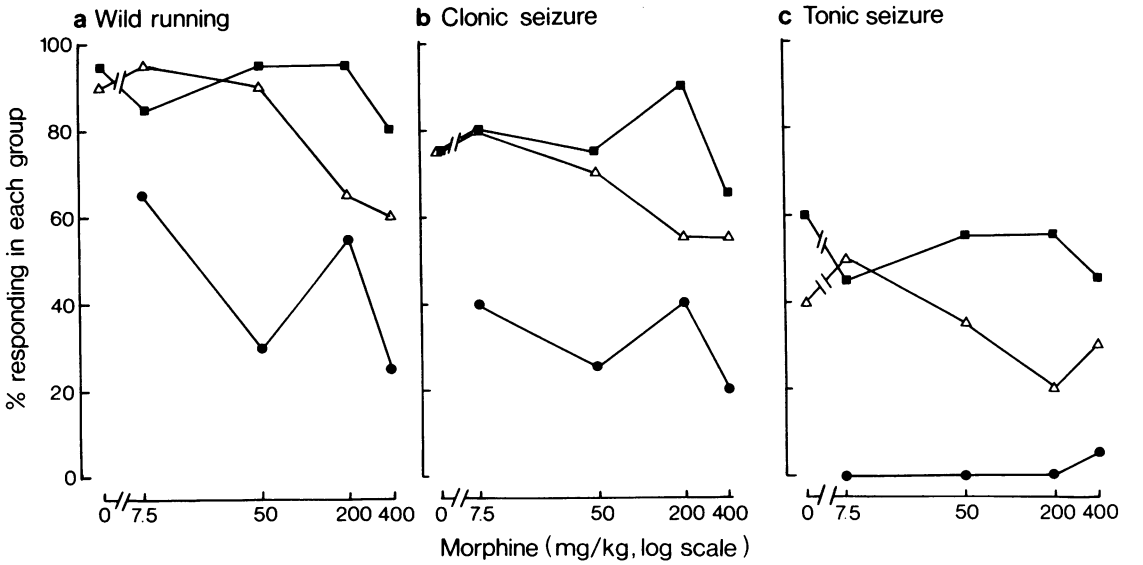

Figure 1 The effect of morphine, and its antagonism by naloxone, on the incidence of three phases of the audiogenic seizure response in BALB/c mice. Naloxone reduced the effect of morphine in a dose-dependent manner, 2 mg/kg returning the incidence of seizure to control levels. (●) Morphine; (△) morphine + 1 mg/kg naloxone; (■) morphine + 2 mg/kg naloxone.

does not significantly antagonize the effects of morphine on latency of wild running and clonic seizure. However, the median latencies of wild running were 4.1, 3.85 and 3.6 s for the morphine, morphine plus naloxone 1 mg/kg, morphine plus naloxone 2 mg/kg groups, respectively (Kruskal-Wallis, $H(2)=2.931$, $P>0.2$). There was a similar trend in the latency of clonic seizure, the medians being 8.5, 8.4 and 7.25 s respectively (Kruskal-Wallis, $H(2)=2.474$, $P>0.2$).

Discussion

Our results show that morphine can suppress the incidence and severity of priming-induced audiogenic seizures in normally seizure-resistant BALB/c mice (Chen, 1973). Naloxone can reverse this suppression of seizures by morphine and thus suggests that morphine is acting via a specific receptor.

The results presented here differ from the negative findings of Bentley (1961) who used rats, and Collins & Horlington (1969) who used a spontaneously susceptible strain of mice and measured only complete abolition of the wild running component. Similarly Boggan (1973) found that morphine did not suppress priming-induced seizures in C57 BL/6 mice. This discrepancy between C57 BL/6 mice used by Boggan and our results cannot be accounted for by dosage differences but may be due to strain differences as there is some evidence for different strain susceptibility to morphine (Gebhart & Mitchell, 1973; Oliverio & Castellano, 1974).

This seizure test may provide a useful model for the study of tolerance and physical dependence development, particularly since it has been shown that alcohol (Freund & Walker, 1971) and barbiturate (Gates & Chen, 1974; Bentley, 1961) withdrawal can induce seizures in some resistant strains of mice and rats.

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