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BRIEF COMMUNICATION

Noncompetitive Antagonism of Morphine Analgesia by Diazepam in the Formalin Test

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ABBOTT, F V AND K B J FRANKLIN *Noncompetitive antagonism of morphine analgesia by diazepam in the formalin test* PHARMACOL BIOCHEM BEHAV **24**(2) 319–321, 1986 —The effects of diazepam on morphine analgesia dose effect curves in the formalin test and in two forms of the tail flick test were examined In one form of the tail flick test the animals were restrained in wire restrainers (a stressful procedure) while in the other they were left free and briefly hand-held for testing Morphine analgesia in the restrained form of the test is known to depend on raphe magnus 5HT projections to the spinal cord while the other tests do not involve this system Diazepam (0 2 and 1 0 mg/kg) noncompetitively antagonized morphine analgesia in the formalin test but had no effect on morphine analgesia in the tail flick test It is concluded that diazepam does not antagonize morphine analgesia through its antianxiety action reducing the serotonergic response to stress It is suggested that the sensitivity of the formalin test to diazepam antagonism of morphine analgesia may be of clinical significance since formalin test pain resembles postoperative pain in humans

Morphine Diazepam Analgesia Tail flick test Formalin test Stress

BENZODIAZEPINES (BDZs) are often used together with narcotic analgesics but the interactions of these drugs are not well understood Several groups of investigators have reported that diazepam, chlordiazepoxide and other BDZs antagonize the analgesic effect of morphine or meperidine in animal models of pain [7, 14, 23] Others have not found any reliable effect of diazepam on morphine analgesia [18,20] while two studies report that chlordiazepoxide potentiates morphine analgesia [7,8]

One reason for the inconsistent interaction of BDZs and morphine analgesia may lie in the different testing situations employed by different laboratories Recently it has been shown that there are at least two types of morphine analgesia found in animal tests One type, exemplified in rats that are restrained during tail flick testing, is sensitive to an interaction between morphine and brain 5HT, the level of which is elevated by restraint stress [13] This type of analgesia depends on raphe magnus 5HT fibres projecting to the spinal cord dorsal horn [3,12] In the tail flick test with unstressed animals [12] and in the formalin test, which measures a freely moving animal's response to a minor tissue injury produced by a subcutaneous injection of dilute formalin [6], analgesia does not depend on serotonergic raphe projections to the spinal cord ([1,2], Watkins, L R , personal communication)

Since BDZs are efficacious anxiolytic agents they may antagonize morphine analgesia by reducing the effects of situational stress If this were so, BDZ should antagonize morphine analgesia in the restrained tail flick test rather than in the formalin test or the tail flick test with unstressed animals To examine this possibility and explore the nature of BDZ antagonism of morphine analgesia we established dose-effect relations for morphine analgesia with and without concurrent diazepam treatment in the formalin test and the restrained and unrestrained tail flick tests

METHOD

Male Long Evans hooded rats were assigned, in groups of 3 to 5, to be injected SC with morphine sulphate (2–8 mg/kg) alone or in combination with an IM injection of diazepam (0 2–1 0 mg/kg) Each rat was tested once only Diazepam was injected 15 min after morphine Formalin was injected immediately after diazepam Pain scores in the formalin test were averaged over the period 30 to 45 min after morphine Tail flick tests were given 40 min after morphine A pilot study showed that, in the formalin test, maximum interaction between diazepam and morphine occurred with drug injections at these times

Preliminary tests showed that the combination of an opioid and diazepam produced so much sedation that pain rating was difficult Since it is known that tolerance to morphine does not alter the pharmacokinetics of morphine [11] or opiate receptor binding [9] it was decided to pre-adapt rats to the opioid for 6 days preceding the test (2 days at 4 mg/kg,

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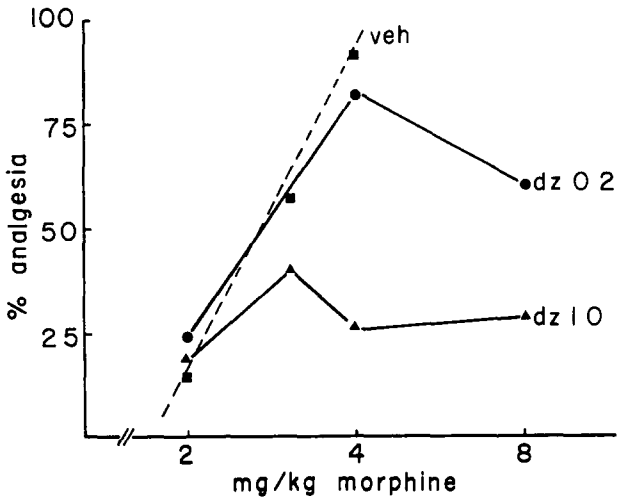

FIG 1 Dose response curves for morphine analgesia in the formalin test in rats treated with 0 2 mg/kg diazepam (solid line and circles), 1 0 mg/kg diazepam (solid line and triangles) or with the diazepam vehicle (dotted line and squares) In diazepam treated rats the morphine dose effect curve was not well fitted by a regression line and solid lines connect raw data points

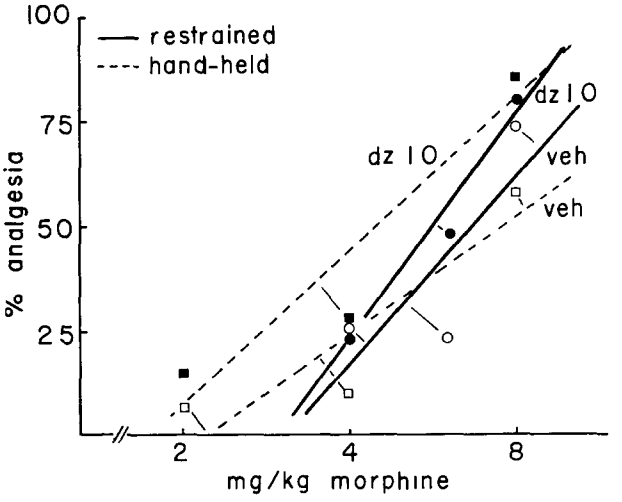

FIG 2 Dose response curves (regression lines) for morphine analgesia in tail flick test in rats tested restrained (solid lines and circles) or unrestrained (dotted lines and squares) Filled symbols represent groups of animals tested after diazepam (1 0 mg/kg) pretreatment and open symbols represent groups tested after injections of the diazepam vehicle

4 days at 8 mg/kg) Adaptation was carried out in the room where the experiment was to be done and rats to be formalin tested were placed in the apparatus for 30 min per day

Formalin Test

Formalin (0 05 ml of 2 5%) was injected SC into the plantar surface of one hind paw of each rat The rat was then placed in a Plexiglas viewing chamber and, beginning 15 min after injection, behavior was rated for 15 min

The experimenter recorded the amounts of time the rat spent walking firmly on the injured paw (pain=0), partially elevated or favoured the paw (pain=1), elevated the paw (pain=2), or licked and chewed at the paw (pain=3)

Tail Flick Test

Responses to a thermal pain stimulus were assessed by the latency with which a rat removed its tail from 55°C water Maximum latency was set at 20 sec In the restrained form of the test rats were placed in wire restraining tubes from 10 min before drug injection till the end of the test In the unrestrained form of the test rats were left free in their home cages (3–4 rats per cage) and handheld during each test for approximately 30 sec

Data Analysis

Analgesia was expressed as percent of maximal effect by the formula

$$\frac{(\text{observed score} - \text{minimum score}) \times 100}{\text{maximum score} - \text{minimum score}}$$

Maximum score was 20 sec for the tail flick test and 0 for the formalin test Minimum scores were the undrugged control averages of 2 5 sec and 2 2 respectively

Drug effects were plotted against log dose and least squares regression lines fitted to the steeply rising portion of the dose effect curves An AD50 was defined as the dose at the half-maximal effect An AD50 and its standard error was computed by jackknifing the estimates of the dose producing half-maximal analgesia [17]

RESULTS

In the formalin test (Fig 1) diazepam 1 0 mg/kg markedly attenuated morphine analgesia and significantly reduced the slope of the morphine dose effect relation, $t(26)=4\ 416$, $p<0\ 025$ Diazepam 0 2 mg/kg also slightly reduced the asymptote of the morphine dose effect relation but the change in slope did not reach significance, $t(24)=1\ 19$, NS In controls tested without morphine, diazepam (1 0 mg/kg) did not alter the formalin pain rating ($U=6$, $n1=n2=4$, $p=0\ 343$, Mann Whitney) Though these animals appeared sedated, they continued to protect the formalin-injured paw even when lying down Morphine increased the sedation but most animals still protected the injured paw even with 8 mg/kg morphine With 16 mg/kg morphine plus 1 0 mg/kg diazepam animals were so sedated they appeared unable to stand on three legs so data for this dose were discarded Nevertheless, when disturbed, most animals showed clear signs of pain in the injured paw

Morphine dose effect curves for the tail flick tests are shown in Fig 2 Diazepam did not alter the slope of the dose effect relation in either the handheld, $t(26)=0\ 88$, NS, or the restrained, $t(22)=0\ 36$, NS, form of the test In both cases the AD50s for diazepam treated rats lay within the 95% confidence interval for the AD50 for morphine alone Both with and without diazepam the slope of the morphine dose effect relation was steeper when animals were restrained than when they were handheld, $t(24)=2\ 29$ and $t(24)=2\ 36$ respectively, $p<0\ 05$

DISCUSSION

These results show that low doses of diazepam can antagonize the analgesic effect of morphine in the formalin test

and indicate that the antagonism is noncompetitive Diazepam itself did not alter formalin pain scores

The data for the tail flick test are consistent with a previous report that diazepam does not significantly affect morphine analgesia in the rat [18] One study that did find antagonism of morphine in the rat [14] used intracerebral injections of diazepam so the effective dose levels may have been higher than those obtained in the present study Studies which report benzodiazepine antagonism of morphine analgesia in the mouse have used much larger doses of diazepam (AD50 2 1 mg/kg) [7,19] or chlordiazepoxide [19,23] Shannon *et al* [20] found no effect of 1 0 mg/kg diazepam on the dose effect curve for morphine analgesia in the mouse This result is in complete agreement with our present data for the rat tail flick test The data for diazepam is consistent in showing that low doses (1 0 mg/kg or less) do not alter morphine analgesia in the tail flick test while above 2 0 mg/kg diazepam antagonizes morphine The nature of the antagonism is not established for the tail flick test though we have here found that in the formalin test the antagonism seems to be noncompetitive

The results did not support the hypothesis that diazepam antagonizes analgesia by reducing stress potentiation of morphine analgesia In this experiment restraint stress increased the slope of the morphine dose-effect relation and diazepam did not alter this effect Moreover, the formalin test, which is insensitive to the tryptaminergic mechanism that underlies restraint potentiation of morphine analgesia [1, 2, 12], was most sensitive to antagonism of morphine analgesia by diazepam

The fact that morphine analgesia in the formalin test seems to be particularly sensitive to diazepam may be of clinical significance Antagonism was found with low doses of diazepam that are nevertheless effective in antianxiety assays [5,22], i e , doses which, allowing for species differences in pharmacodynamics, are comparable to those used clinically Furthermore, the formalin test pain may resemble pain for which opiates are used in humans more than most animal tests Formalin pain results from tissue injury, is relatively long lasting (1 to 2 hours), and experimenters who have experienced it describe it as poorly localized, burning and aching [6] These characteristics are typical of pain experienced by patients after injury [4,16] Benzodiazepine antagonism of morphine analgesia has not apparently been observed clinically [10,18] However the present findings suggest that benzodiazepines may reduce the maximum analgesia obtainable while having little effect on low levels of analgesia Recent studies of clinical practice are consistent in finding that patients who do receive opiate medication for pain usually receive minimal rather than optimal doses [15,21] Thus any effect of concurrent benzodiazepine treatment would be difficult to detect

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