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Effect of Chronic Morphine on the Response to and Disposition of Other Drugs[1,2,3,4]

R. A. HOWD AND G. T. PRYOR

SRI International, Menlo Park, CA 94025

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HOWD, R. A. AND G. T. PRYOR. *Effect of chronic morphine on the response to and disposition of other drugs.* PHARMAC. BIOCHEM. BEHAV. **12**(4) 577–586, 1980.—Male Fischer rats were implanted SC with a total of six 75-mg morphine (base) pellets over 8 days and tested on the 11th day. On Day 11 a dose of 0.15 mg/kg of naloxone precipitated strong withdrawal signs. Chronic treatment with morphine by this schedule inhibited weight gain but other physical effects were minimal. When tested on Day 11, the morphine-pelleted rats performed a pole-climb conditioned avoidance response (CAR) task as well as placebo-pelleted rats, either because they had become tolerant to the CAR-impairing effect of the residual levels of morphine or because the residual levels of morphine were too low to affect the response. However, the impairing effects on CAR performance of pentobarbital, phenobarbital, thiopental, barbital, diazepam, and ethanol were all prolonged and/or exaggerated by the morphine pretreatment. This increase in the depressant effects of these drugs could not be completely accounted for by the effect of morphine on their uptake, distribution, or elimination. Although an interaction between the drugs and residual levels of morphine could not be ruled out, these results suggest the possibility that chronic treatment with morphine may have caused an increase in the central responsiveness to these drugs as well.

Chronic morphine Ethanol Conditioned avoidance response Diazepam Barbiturates Drug interaction

CHRONIC treatment with narcotics causes a number of physiological changes in humans and laboratory animals. Development of tolerance and physical dependence are the best known of these alterations, but others are of theoretical and practical interest and importance. For example, chronic treatment of rats and mice with morphine prolongs the sedative effect of pentobarbital [10] and hexobarbital [2,15]. This effect is associated with an inhibition of drug-metabolizing enzymes and consequent slower elimination of these drugs [1, 2, 4, 5, 10–15, 17]. Bousquet *et al.* [2] also reported that meprobamate-induced sleeping time (but not barbital-induced sleeping time) was prolonged by repeated treatment with morphine.

The purposes of the experiments reported here were to: (1) confirm and extend these findings by examining the behavioral response to a series of barbiturates—pentobarbital, phenobarbital, thiopental, and barbital—after chronic treatment with morphine; (2) compare similarly the responses to two other drugs with sedative effects—diazepam and ethanol—but unrelated to the barbiturates; (3) determine the effect of chronic treatment with morphine on the uptake and disposition of one member of each drug class—pentobarbital, diazepam, and ethanol; and (4) compare the effect of pentobarbital on the behavioral response after a single injection of morphine with that seen after chronic treatment with morphine when the rats were tolerant to the impairing effect of the residual levels of morphine alone.

METHOD

Animals

Male Fischer rats, 55 to 60 days old (150 to 160 g) from Simonsen Laboratories, Gilroy, California, were housed singly in wire mesh cages with Purina rat chow and water available ad lib. The rats were maintained at a temperature of 22°C with lights on at 0700 hours and off at 1900 hours.

Apparatus

The behavioral tests were carried out in 30.2×30.2×35.6 cm Plexiglas chambers with brass rod floors through which a scrambled, constant-current shock could be applied. A 1.27-cm-diameter aluminum pole was suspended from the center of the ceiling, downward displacement of which signaled a response. Conditional stimuli (CS) were provided by an oscillating (2.5 sec) increase in the intensity of the house light, or a 4-kHz tone, or a 120-μA nonaversive current applied through the floor. Each of 24 such chambers was housed inside a separate sound-attenuated cubicle in a plywood cabinet. The chambers were interfaced with a Digital Equipment Corporation PDP 8/F computer located in an

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[2]Gratitude is expressed to F. Larsen, T. Steeger, J. Trainer, and J. Kalivoda for their skillful assistance in carrying out this work.

[3]Naloxone HCl was a generous gift from Endo Laboratories.

[4]Partial results were presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics held in Houston, TX, 17 August 1978.

adjoining room that provided automatic stimulus presentation and data collection.

Training

Rats were given 30 trials to learn to escape a 1-mA foot shock by climbing or pulling a 20-cm pole. Each trial lasted 20 sec unless terminated earlier by the rat's response. The trials were presented randomly in time but every 90 sec on the average (20 to 120 sec range). Then the rats were given three daily 60-trial sessions to learn to avoid the aversive foot shock by climbing or pulling a 13-cm pole in the presence of the CS. The three CS were presented in random order (one on each trial) and preceded the aversive foot shock by 10 sec. In the absence of a response the CS and the aversive foot shock remained on for an additional 20 sec. If the rat did not respond until the aversive foot shock was initiated, an avoidance failure was recorded. If the rat did not respond throughout the trial, an escape failure was recorded. At the end of training most rats avoided the aversive foot shock on 80% or more of the trials. Rats that failed to learn the escape response were discarded (less than 5% with this strain).

Design

For evaluating the acute effects of morphine and pentobarbital, and their interaction, 72 rats were trained to perform the CAR as described above. On the next day they were given nine warm-up trials after which they were injected IP with saline or 2, 4 or 10 mg/kg morphine HCl (2 ml/kg in saline) (N=18 per group). Fifteen minutes later half the rats in each group were injected IP with saline and the other half with 15 mg/kg of sodium pentobarbital (2 ml/kg in saline) and given a 60-trial test for CAR performance administered the same as during training. The CAR test began within 15 min after the second injection and lasted about 2 hr with trials being presented at the rate of one every 2 min on the average.

For each experiment designed to evaluate the response to other drugs after chronic treatment with morphine, separate groups of rats (usually 72) were trained to perform the CAR as described above. Then half the rats in each group had placebo pellets implanted and the other half had morphine pellets implanted. The pellets were implanted subcutaneously on the back just below the neck under light ether anesthesia. One pellet was implanted on Day 1, two pellets on Day 4, and three pellets on Day 8. This schedule was used because preliminary experiments showed that it induced moderate to high tolerance to and physical dependence on morphine without being excessively toxic. The rats within each chronic treatment group were then assigned randomly to 1 of 4 groups that would receive vehicle or 1 of 3 doses of the test drug. On Day 11 all rats were given nine warmup trials followed by administration of the appropriate vehicle or the assigned dose of the test drug. A 60-trial test for CAR performance was then administered as in the acute experiment. Except for ethanol, which was administered orally, all test drugs and their vehicles were injected IP. For ethanol, the rats were fasted overnight before the test, otherwise all rats had free access to food and water throughout the experiments. Body weights were recorded periodically throughout each experiment but at least on each morning before the pellets were implanted and on the morning of the test day.

For evaluating the disposition of ^{14}C-pentobarbital, ^{14}C-diazepam, and ethanol after chronic treatment with morphine, separate groups of untrained rats were implanted with placebo or morphine pellets according to the same schedule as for the behavioral experiments. On Day 11 all rats were given a single dose of the test drug. Separate groups of placebo-pelleted and morphine-pelleted rats (usually six per group) were then sacrificed at selected intervals for determination of the levels of the test drug in plasma, brain, and liver by the methods described below. Ethanol was given orally after an overnight fast. ^{14}C-pentobarbital and, in one experiment, ^{14}C-diazepam were injected IP. In another experiment ^{14}C-diazepam was injected IV in the tail vein and plasma was sampled by retro-orbital puncture at selected times thereafter in the same rats. Before sacrifice in these experiments each rat was judged as to the degree of sedation caused by the test drug. The subjective scale used was: 0=no sedation, 1=mild sedation, 2=moderate sedation, and 3=unconscious.

To determine the extent to which this schedule of chronic treatment had induced physical dependence on morphine, separate groups of rats were implanted with placebo (N=10) or morphine (N=30) pellets according to the same schedule as described above. On Day 11 they were injected SC with 0.15, 0.3, or 0.6 mg/kg naloxone HCl. Body weight and body temperature were recorded before and 15, 30, and 60 min after naloxone. The rats were observed for 30 min after naloxone for signs of precipitated withdrawal, including wet shakes, unprovoked vertical jumping, running bursts, writhing, teeth chattering, rhinorrhea, lacrimation and diarrhea.

Analysis of Drugs in Plasma and Tissues

The rats were sacrificed by decapitation after which blood was drained from the carcass, heparinized, and centrifuged for collection of plasma. Brain and liver were removed and frozen on dry ice for subsequent weighing, then thawed and homogenized with a Polytron in two volumes of water. One-tenth ml aliquots of the plasma and homogenates were counted in a Searle Mark III scintillation counter in 10 ml of Scintosol or taken for ethanol assay. For assay of pentobarbital and diazepam, trichloracetic acid (TCA) was added to other aliquots of tissue homogenates or plasma to a final concentration of 5% in order to precipitate proteins and release drug from protein binding sites. After centrifugation, the supernatant was extracted with an organic solvent to isolate and determine the relative concentrations of intact drug and nonpolar metabolites in the tissues.

For the experiment in which ^{14}C-diazepam was injected IV, two 70-μl samples of blood were obtained at each sampling time in heparinized capillary tubes by retro-orbital puncture from rats lightly anesthetized with CO_2. The tubes were centrifuged in an IEC microcapillary centrifuge, 30 μl of plasma were removed from each, and these samples were combined for scintillation counting in 10 ml of Scintosol. At two hours, these rats were decapitated and the tissues were homogenized and counted as above.

Pentobarbital was extracted from TCA supernatants by shaking once with three volumes of chloroform or twice with three volumes of toluene. Appropriate aliquots of the organic phase were counted in 10 ml of Scintosol. With both solvents, 90% or more of the ^{14}C extracted from tissues was in the form of intact ^{14}C-pentobarbital. Recovery of ^{14}C-pentobarbital added to homogenates was approximately 100% with toluene and 80% with chloroform.

Diazepam was extracted twice with four volumes of toluene and 0.1- to 1.0-ml aliquot of the extract was counted. This extraction gave 90 to 100% recovery of added

diazepam and, at 2 or more hours after drug injection, a significant extraction of lipophilic metabolites of diazepam.

The chemical identity of the extracted ^{14}C was evaluated by thin-layer chromatography (TLC) on silica gel plates with a solvent system of ethyl acetate:methanol:NH_4OH (85:5:2.5) for both drugs, by comparison with the R_f of authentic standards. Autoradiography was used for localization and to provide a rough estimate of the amounts of ^{14}C in the various areas representing the parent drug and its metabolites.

Counts-per-min per tissue aliquot were converted to μg/g or μg/ml equivalents of the original injected drug, and are presented in this form. The actual percentage of the intact drug moiety varied in each tissue and at each time point with the relative amounts of metabolites increasing with time, especially in the liver.

Ethanol was assayed by a standard enzymatic method using yeast alcohol dehydrogenase [16].

Drugs

Pellets containing 75 mg of morphine base or placebo pellets without morphine were formulated according to the method of Gibson and Tingstad [7]. When administered parenterally, morphine HCl (Penick) was dissolved in saline and injected IP in a volume of 2 ml/kg. Naloxone HCl (Endo Laboratories) was dissolved in saline and injected SC in a volume of 2 ml/kg. Sodium pentobarbital (Sigma) was dissolved in saline and injected IP in a volume of 2 ml/kg. For determining the tissue concentrations of pentobarbital, ^{14}C-pentobarbital (ring-2-^{14}C, 56 mCi/mmole, New England Nuclear) was added to unlabeled drug to achieve a ^{14}C dose of 25 μCi/kg. Diazepam was dissolved in 0.01 N HCl for IP injection in a volume of 4 ml/kg; for IV injection it was dissolved in 40% propylene glycol plus 10% ethanol in 0.05 N sodium benzoate at pH 7.4 in a volume of 1 ml/kg. For determining tissue concentrations of diazepam, ^{14}C-diazepam (2-^{14}C, 57 mCi/mmole, Amersham-Searle) was added to unlabeled drug to achieve a ^{14}C dose of 25 μCi/kg. Ethanol (Gold Shield, IMC Chemical Group, Inc.) was diluted with distilled water and administered orally at a constant volume of 10 ml/kg. Sodium phenobarbital (Mallinckrodt) and sodium thiopental (Abbott) were dissolved in saline and injected IP in a volume of 2 ml/kg. Barbital (Sigma) was dissolved in 5% Tween-80 saline and injected IP in a volume of 2 ml/kg. The doses used are indicated in the results. Doses are given as the salt for all complexed drugs.

Data Analysis

For each behavioral experiment the percentage of avoidance failures and the percentage of escape failures were calculated for each block of ten trials (lasting approximately 20 min) and constituted a within-subjects factor in a mixed factors experimental design. The between-subjects factors for the acute experiment were the dose of morphine and treatment with saline or pentobarbital. The between-subjects factors for the chronic experiments were the chronic treatment (placebo or morphine pellets) and the dose of the test drug. The data are presented as means and their associated standard errors. Results of the analyses of variance of the behavioral data are not presented to avoid congestion in the text and because the magnitude of most of the effects observed and their internal consistency were such that statistical verification was considered unnecessary. Nevertheless, where the reliability of a specific effect is not clearly evident,

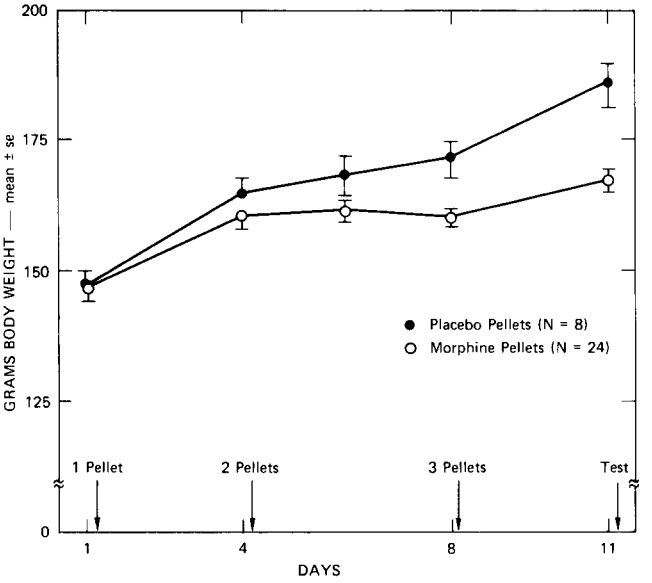

FIG. 1. Body weights of placebo-pelleted and morphine-pelleted rats from a representative experiment.

the results of comparisons using Student's *t*-test or the Mann-Whitney U test [21] are reported. In all cases the criterion for rejection of the null hypothesis was $p<0.05$.

The dispositional data are presented as the means from 5 to 12 rats for each tissue and time point (± SE). The statistical significance of differences between placebo and morphine-pelleted rats was assessed by Student's *t*-test. The ethanol data were further subjected to linear regression analysis, including determination of the variances in the slopes of the tissue and plasma elimination curves.

RESULTS

Mortality, Body Weights and Organ Weights

Mortality caused by this schedule of chronic treatment with morphine was less than 5% throughout this series of experiments. A few morphine-pelleted rats exhibited a dark exudate around the genital area and developed what appeared to be atrophy and/or inflammation of the penis. However, most of the morphine-pelleted rats appeared healthy and did not display any other gross signs of toxicity.

Figure 1 shows results from a typical experiment and illustrates that chronic treatment with morphine by this schedule of pellet implantation inhibited weight gain compared with placebo-pelleted controls. In the experiment shown, the difference in body weights between placebo-pelleted and morphine-pelleted rats was 6% by Day 8, which was statistically significant; the difference on Day 11 was 11%.

Data from those experiments in which the rats were sacrificed on Day 11, showed that the livers from the morphine-pelleted rats typically weighed significantly less as a percentage of body weight (3.48±0.05% in this experiment) than the livers from the placebo-pelleted rats (3.93±0.05%). However, there were never any significant differences in brain weight.

Physical Dependence

The results in Tables 1 and 2 show that this schedule of

TABLE 1

CHANGES IN BODY WEIGHT AND BODY TEMPERATURE IN PLACEBO-PELLETED AND MORPHINE-PELLETED RATS DURING PRECIPITATED WITHDRAWAL AS A FUNCTION OF DOSE OF NALOXONE

		Pretreatment							
				Morphine pellets					
	Time after naloxone (HCl)	Placebo pellets*		Dose of naloxone (mg/kg) 0.15		0.3		0.6	
Measure	(min)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body weight	15	−2.7	0.52†	−6.4	0.66	−7.0	0.70	−7.8	0.58
	30	−3.4	0.51	−7.7	0.57	−7.3	1.03	−9.1	0.33
	60	−3.6	0.50	−8.3	0.52	−9.7	0.75	−10.6	0.63
Body temperature	15	1.9	0.59	−5.6	0.50	−5.0	0.59	−5.5	0.58
	30	2.4	0.46	−6.0	0.36	−6.2	0.37	−8.0	0.61
	60	0.9	0.48	−4.5	0.52	−6.4	0.38	−8.8	0.62

*Two or three rats each were administered each dose of naloxone. There were no differences and the data were combined.

†Values are the mean (± SE) percentage of change from measurements taken just before administration of naloxone. The mean (± SE) body weights of placebo-pelleted and morphine-pelleted rats were 186 (± 3.0) g and 168 (± 1.6) g, respectively; the corresponding body temperatures were 38.2 (± 0.15)°C and 38.8 (± 0.01)°C, respectively. The means for all morphine-pelleted groups of rats were significantly different from those of the placebo-pelleted rats at each time for both measures.

TABLE 2

WITHDRAWAL SIGNS SEEN IN PLACEBO-PELLETED AND MORPHINE-PELLETED RATS AFTER NALOXONE

	Pretreatment							
			Morphine pellets					
	Placebo pellets*		Dose of naloxone (mg/kg) 0.15		0.3		0.6	
Withdrawal sign	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wet shakes†	0.5	0.33	7.4	1.16§	8.5	2.12§	3.5	0.68§
Vertical jumping†	0.2	0.25	0.5	0.27	1.4	0.42§	2.5	1.05§
Running bursts†	0.5	0.27	2.8	1.46	5.1	2.62§	9.4	4.29§
Writhing†	0.0	0.00	4.4	1.25§	6.4	1.67§	5.9	1.43§
Teeth chattering†	0.2	0.16	3.8	0.80§	3.1	0.48§	3.5	0.50§
Rhinorrhea‡	1/8		5/8		7/8		3/8	
Lacrimation‡	1/8		4/8		5/8		4/8	
Diarrhea‡	0/8		8/8		8/8		7/8	

*Two or three rats each were administered each dose of naloxone. There were no differences and the data were combined.

†Total incidence observed during the 30-min observation period.

‡These signs were recorded as present or absent. The data shown are the numbers of rats displaying the sign out of the eight tested.

§$p<0.05$ compared with placebo-pelleted controls by the Mann-Whitney U test.

pellet implantation induced a moderate to high degree of physical dependence on morphine as evidenced by significant decreases in the body weights and body temperatures of the morphine-pelleted rats compared with controls after naloxone, and the appearance of various other signs and symptoms characteristic of naloxone-precipitated withdrawal. The lowest dose of naloxone tested (0.15 mg/kg) was effective in this regard.

Acute Effect of Morphine and Its Interaction with Pentobarbital

Figure 2 shows that a dose of 10 mg/kg morphine HCl alone caused a significant increase in avoidance failures without any appreciable number of escape failures. This effect of morphine was maximal during the first block of ten trials and decreased thereafter. Recovery was complete by

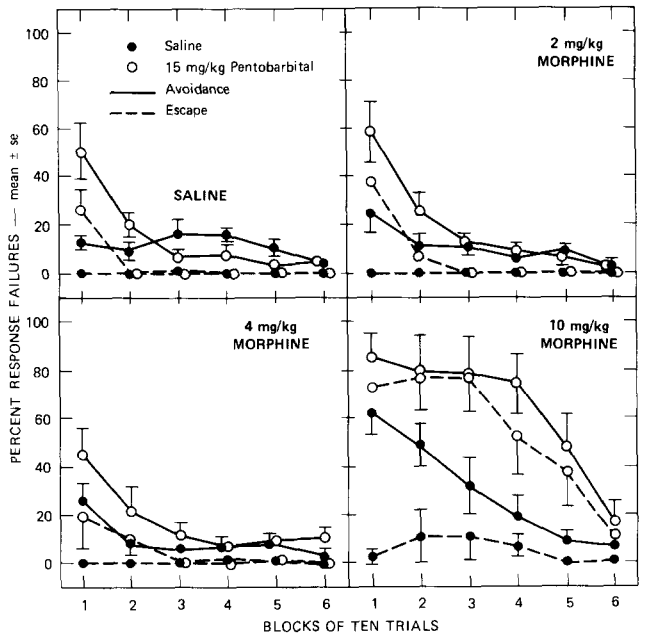

FIG. 2. Acute interaction between morphine HCl and pentobarbital as measured by performance of a conditioned avoidance response. Eight or nine rats were in each group.

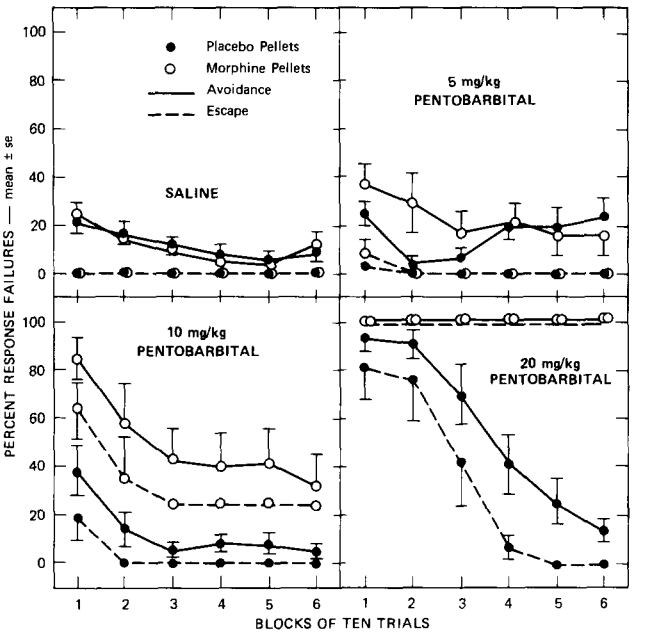

FIG. 3. Effect of pentobarbital on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Eight or nine rats were in each group.

the end of the 2-hr test session. The lower doses of morphine (2 and 4 mg/kg) did not significantly affect either avoidance or escape responses.

The 15-mg/kg dose of pentobarbital alone had a moderate but short-lasting effect on CAR performance. Both avoidance and escape failures were significantly greater than those of controls, but only during the first block of ten trials.

When 15 mg/kg of pentobarbital was given just after the two lower doses of morphine HCl, the impairment of CAR performance was not significantly different from that seen after pentobarbital alone. When the dose of morphine HCl (10 mg/kg) alone was sufficient to cause a marked increase in avoidance failures, its combination with 15 mg/kg pentobarbital significantly prolonged the impairment beyond that expected from a simple addition of these doses of either drug alone.

Response to and Disposition of Pentobarbital After Chronic Treatment with Morphine

Figure 3 shows that the morphine-pelleted rats performed the CAR as well as placebo-pelleted rats when tested on Day 11 (upper left-hand panel). Nevertheless, pentobarbital impaired the performance of the morphine-pelleted rats significantly more than that of the placebo-pelleted rats except for the lowest dose of pentobarbital (5 mg/kg), although the same trend was apparent at that dose as well. The higher doses of pentobarbital (10 and 20 mg/kg) caused moderate to marked initial impairment in placebo-pelleted rats, but the magnitude and duration of this effect was much greater in the morphine-pelleted rats. The latter rats given 20 mg/kg pentobarbital did not avoid or escape foot shock on a single trial throughout the test session.

Figure 4 shows that the behavioral impairment caused by pentobarbital in the morphine-pelleted rats was accompanied by higher levels of total ^{14}C derived from ^{14}C-pentobarbital in plasma, brain, and liver than in placebo-pelleted controls.

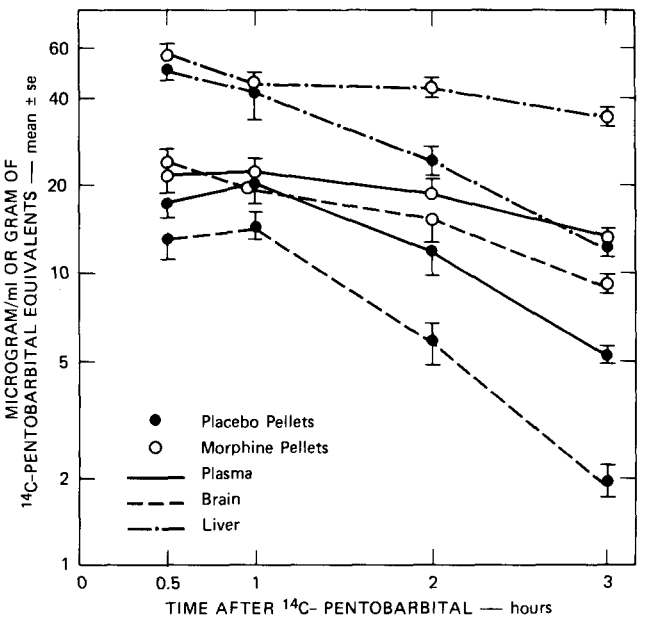

FIG. 4. Disappearance of total ^{14}C derived from ^{14}C-pentobarbital from plasma, brain, and liver of placebo-pelleted and morphine-pelleted rats. The dose of ^{14}C-pentobarbital was 15 mg/kg (25 μCi/kg). Six rats were in each treatment group sacrificed at each time point.

The differences in brain were significant at all time points sampled (0.5 to 3.0 hr). The differences in plasma and liver were significant at 2 and 3 hr. Moreover, the rates of disappearance of total ^{14}C from plasma, brain, and liver were slower in morphine-pelleted rats than in placebo-pelleted rats. Although not shown, toluene-extractable ^{14}C was also

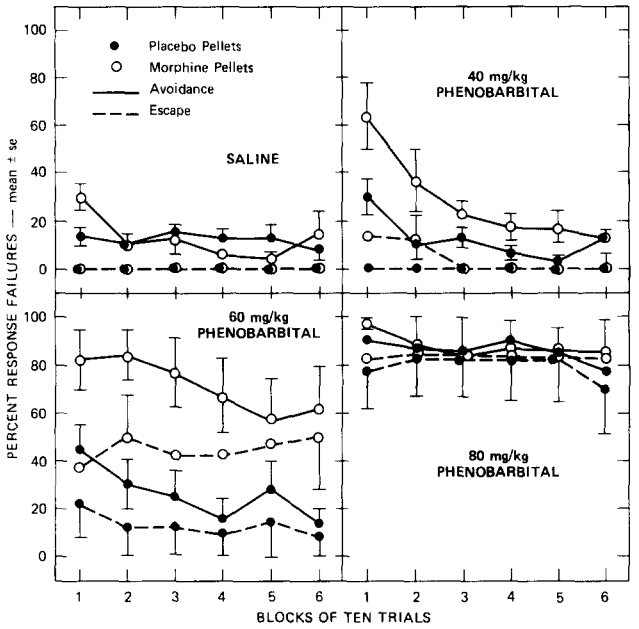

FIG. 5. Effect of phenobarbital on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Five or six rats were in each group.

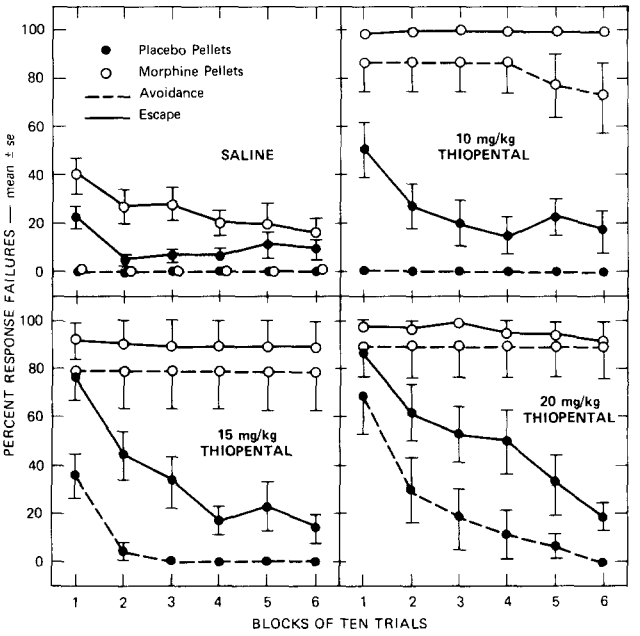

FIG. 6. Effect of thiopental on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Nine rats were in each group.

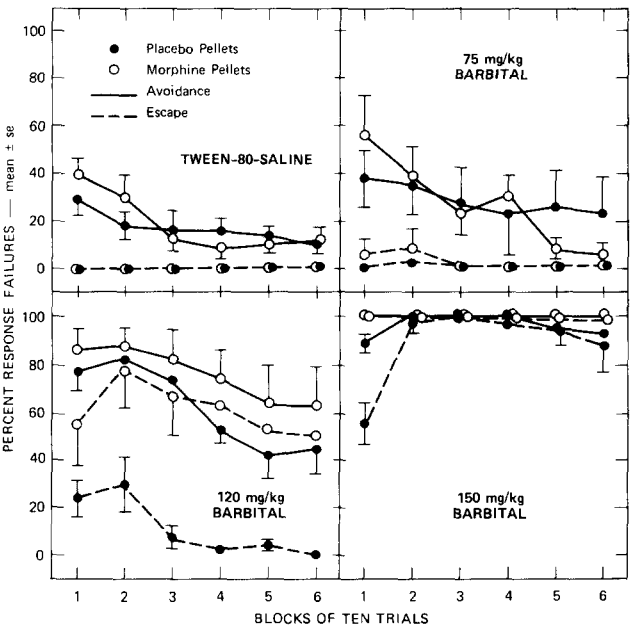

FIG. 7. Effect of barbital on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Five or six rats were in each group treated with barbital. Nine rats were in the groups treated with vehicle.

higher in the brains of morphine-pelleted rats than in placebo-pelleted rats; TLC and autoradiography showed that almost all of the toluene-extractable ^{14}C in brain was in the form of intact ^{14}C-pentobarbital.

Effect of Chronic Treatment with Morphine on the Response to Phenobarbital, Thiopental, and Barbital

Figure 5 shows that phenobarbital caused a dose-related increase in avoidance and escape failures in placebo-pelleted rats and that this effect was significantly greater in the morphine-pelleted rats. The difference between placebo-pelleted and morphine-pelleted rats was most evident at the intermediate dose of phenobarbital (60 mg/kg). No difference was apparent at the highest dose of phenobarbital (80 mg/kg) because it caused almost complete elimination of CAR performance in both groups.

Figure 6 shows that thiopental caused a clear dose- and time-related impairment of CAR performance in placebo-pelleted rats. The rats recovered from this effect fairly rapidly. However, in the morphine-pelleted rats both the avoidance and the escape responses were severely impaired by all doses of thiopental and there was no significant recovery throughout the test session.

Barbital-induced impairment of the CAR was not as strongly affected by chronic treatment with morphine as it was for the other three barbiturates, even though the same trend was evident for the 120 mg/kg dose (Fig. 7). However, escape failures were markedly increased by 120 mg/kg of barbital in the morphine-pelleted rats compared with the placebo-pelleted rats. The highest dose of barbital (150 mg/kg) effectively eliminated CAR performance throughout the test session for both groups. This effect appeared sooner (during the first block of ten trials) in the morphine-pelleted rats than in the placebo-pelleted rats ($p<0.05$ by the Mann-Whitney U-test).

Effect of Chronic Treatment with Morphine on the Response to and Disposition of Diazepam

Diazepam administered IP caused a dose-related, but short-lived, impairment of CAR performance in placebo-pelleted rats (Fig. 8). Even with the highest dose of diazepam (4 mg/kg), which caused fairly marked impairment during the first block of ten trials, recovery was nearly complete by the end of 30 trials (about 60 min). These effects of diazepam

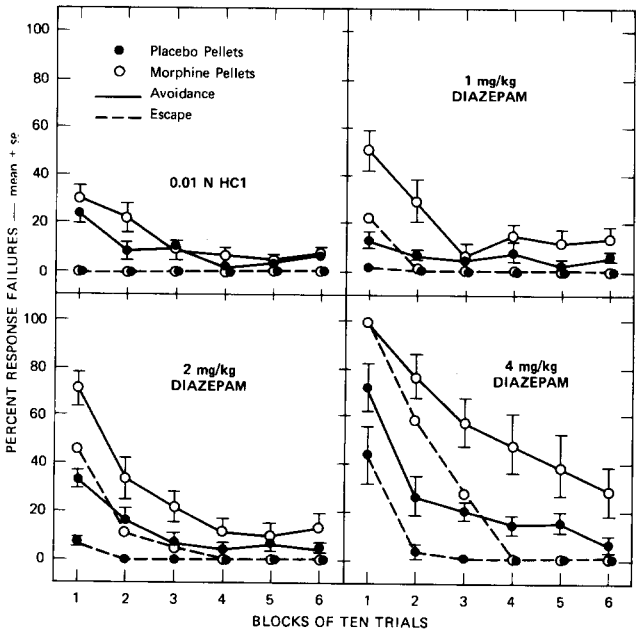

FIG. 8. Effect of diazepam on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Eight or nine rats were in each group.

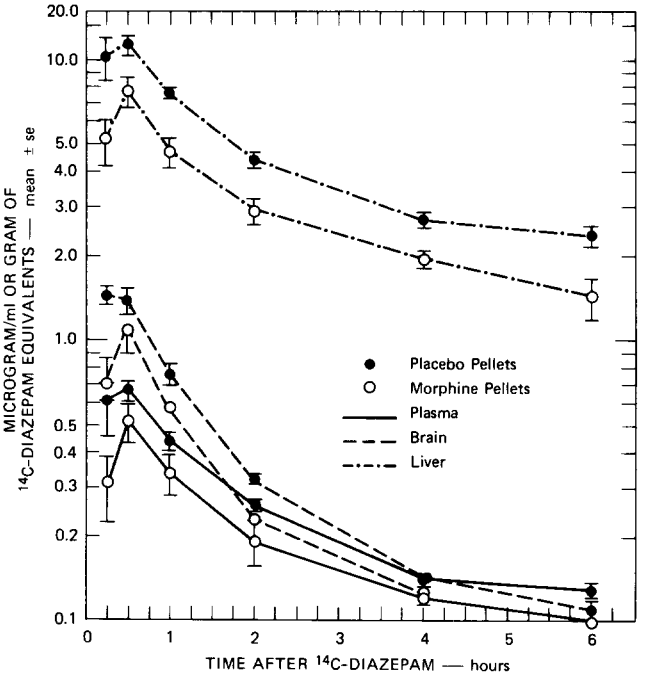

FIG. 9. Disappearance of total ^{14}C derived from ^{14}C-diazepam from plasma, brain, and liver of placebo-pelleted and morphine-pelleted rats.

were greater in the morphine-pelleted rats than in the placebo-pelleted rats in both magnitude and duration of action. The differences between placebo-pelleted and morphine-pelleted rats during the first ten trials were significant for both avoidance and escape responses at all doses of diazepam (all $ps<0.05$ by the Mann-Whitney U-test). Avoidance failures were significantly greater in the morphine-pelleted rats given 4 mg/kg diazepam than in either group of saline-treated controls throughout the test session ($ps<0.05$ for each block of 10 trials), whereas there was no difference in this regard in the placebo-pelleted rats after only 30 trials.

In contrast to the results obtained with ^{14}C-pentobarbital, the levels of ^{14}C-derived from ^{14}C-diazepam were lower in the plasma, brain, and liver from morphine-pelleted rats than in those from placebo-pelleted rats at all times sampled. The data shown in Fig. 9 represent the results from two experiments (5 or 6 rats at each time point) that were essentially identical except for the times when the rats were sacrificed. Some rats in both experiments were sacrificed at 1 and 2 hours and, because there were no significant differences between experiments in the values obtained, the data were combined. The magnitude of the differences in levels of ^{14}C between placebo-pelleted and morphine-pelleted rats was about the same between 1 and 6 hours, and therefore there was no evidence for any change in rate of metabolism caused by chronic treatment with morphine. Although not shown, the same relative results were found for the extracted ^{14}C; disappearance curves were parallel for the two groups, with morphine-treated rats lower in all tissues at each time. TLC and autoradiography indicated that significant amounts of several lipophilic metabolites of diazepam were present in the extracts at the later time points. However, there were no apparent differences in the ratios of metabolites to each other or to diazepam caused by chronic treatment with morphine. The sedation caused by this dose of diazepam was greater in the morphine-pelleted rats than in the placebo-pelleted rats in these experiments.

TABLE 3

LACK OF EFFECT OF CHRONIC MORPHINE TREATMENT ON THE DISAPPEARANCE OF ^{14}C DERIVED FROM ^{14}C-DIAZEPAM AFTER IV ADMINISTRATION

Tissue	Time after ^{14}C-diazepam (min)	Chronic treatment	
		Placebo pellets	Morphine pellets
Plasma	15	0.41 ± 0.03	0.41 ± 0.02
	30	0.29 ± 0.02	0.32 ± 0.01
	60	0.19 ± 0.01	0.21 ± 0.02
	120	0.08 ± 0.01	0.07 ± 0.01
Brain	120	0.13 ± 0.01	0.13 ± 0.02
Liver	120	1.44 ± 0.07	1.22 ± 0.11

^{14}C-Diazepam (25 μCi/kg, 1 mg/kg) was administered IV in the tail vein. Values are the mean (± SE) of six rats at each point. Results are expressed as μg equivalents of ^{14}C-diazepam per ml of plasma or per g wet weight of brain or liver. Plasma was sampled serially in the same rats by retro-orbital puncture. There were no significant differences between morphine-pelleted and placebo-pelleted rats at any time.

The results in Table 3 show that when ^{14}C-diazepam was administered IV, there were no differences between placebo-pelleted and morphine-pelleted rats in the plasma levels of total ^{14}C at any time nor in brain and liver at two hours. However, in agreement with the results found after IP injection of diazepam in the previous experiments, the morphine-pelleted rats were significantly more sedated after IV injection of diazepam than the placebo-pelleted rats.

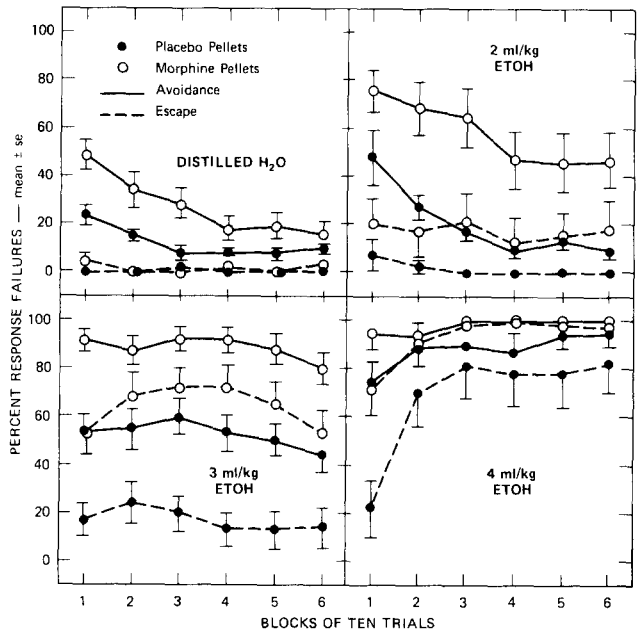

FIG. 10. Effect of ethanol on performance of a conditioned avoidance response by placebo-pelleted and morphine-pelleted rats. Nine rats were in each group.

Effect of Chronic Treatment with Morphine on the Response to and Disposition of Ethanol

Oral administration of ethanol to fasted, placebo-pelleted rats caused a dose- and time-related impairment of CAR performance (Fig. 10). This effect was significantly enhanced by chronic treatment with morphine at all doses of ethanol tested.

The levels of ethanol in brain were not significantly different between placebo-pelleted and morphine-pelleted rats when they were sacrificed 1, 2, or 4 hours after oral administration of 3 ml/kg of ethanol (Table 4). Levels of ethanol appeared to decline more rapidly in the plasma, brain, and liver from morphine-pelleted rats compared with those from placebo-pelleted rats. Linear regression analysis showed that the difference in slopes was significant for plasma, $t(32)=2.54$, $p<0.05$, but not for brain or liver.

TABLE 4

EFFECT OF CHRONIC MORPHINE TREATMENT ON THE DISAPPEARANCE OF ETHANOL FROM PLASMA, BRAIN, AND LIVER

Tissue	Time after ethanol (hrs)	Chronic treatment Placebo pellets	Morphine pellets
Plasma	1	0.34 ± 0.02	0.34 ± 0.01
	2	0.30 ± 0.02	0.25 ± 0.01*
	4	0.20 ± 0.02	0.10 ± 0.02*
Brain	1	0.17 ± 0.02	0.17 ± 0.02
	2	0.13 ± 0.01	0.12 ± 0.01
	4	0.09 ± 0.01	0.05 ± 0.01
Liver	1	0.27 ± 0.02	0.29 ± 0.02
	2	0.19 ± 0.01	0.19 ± 0.01
	4	0.12 ± 0.02	0.07 ± 0.01

Ethanol (3 ml/kg) was administered orally to overnight fasted rats. Values are the mean (± SE) of six rats at each point. Results are expressed as milligrams ethanol per milliliter of plasma or per gram wet weight of brain or liver.

*$p<0.05$ compared with placebo-pelleted rats by t-test.

DISCUSSION

Our results were in agreement with those of Ho *et al.* [10] in showing that chronic treatment with morphine increased and prolonged the action of pentobarbital and that this effect was accompanied by an increase in brain levels of ^{14}C-pentobarbital and a decrease in its rate of disappearance from plasma, brain, and liver. These latter dispositional alterations may have been sufficient to account for a major portion of the exaggerated behavioral response to pentobarbital. However, because the pellets were not removed, it is also possible that residual levels of morphine may have interacted with the pentobarbital to contribute to the effect. We chose not to remove the morphine pellets in these experiments to avoid any complications associated with the rats being in a state of withdrawal at the time of challenge and testing. Because the residual levels of morphine were not measured in these experiments and, therefore, may have been significant [14,18], their contribution cannot be ascertained directly. However, because the morphine-pelleted rats performed the CAR as well as the placebo-pelleted rats at the time of testing, they were either tolerant to the residual levels of morphine alone or the levels were too low to affect this response. Lesher and Spratto, in a similar experiment, assumed that their rats were also tolerant to the sedative effect of the residual levels of morphine, and they were compelled to conclude that "no tolerance develops to potentiation of hexobarbital by morphine ([15] p. 182)." However, although we found that the acute combination of morphine and pentobarbital caused a longer-lasting impairment of CAR performance than would be expected from the simple additive effects of the two drugs, this interactive effect only occurred at a dose of morphine that caused marked impairment by itself. Lower doses of morphine that were ineffective alone did not interact significantly with pentobarbital. Therefore, these results suggest that an acute interaction between residual levels of morphine and pentobarbital was of questionable importance in accounting for the potentiation caused by our schedule of pellet implantation.

It was of considerable interest that not only the impairing effect of pentobarbital on the CAR but also that of phenobarbital, thiopental, and barbital was increased and/or prolonged by chronic treatment with morphine. Phenobarbital is a long-acting barbiturate that is metabolized much slower than pentobarbital [9]. Thiopental is an ultra short-acting barbiturate whose termination of action is generally regarded as being due to tissue redistribution because of its high lipid solubility [9]. Finally, barbital is long-acting, not very lipid soluble, and not metabolized to an appreciable extent by the rat, thus requiring primarily urinary excretion for its termination of action [9].

In our experiment with thiopental, redistribution may not have been the limiting factor in termination of action because the drug was administered IP rather than IV, and the absorption time should be similar to the time it takes to equilibrate in tissues [8]. Nevertheless, thiopental is also metabolized much slower than pentobarbital [9] and, therefore this mechanism would appear to be of less importance in explain-

ing the observed results with this barbiturate than for pentobarbital. Others [3] have reported that thiopental-induced sleeping time and the half life of thiopental were markedly increased by prior acute administration of morphine. If the residual levels of morphine in our experiment similarly increased the half life of thiopental in brain, then this mechanism could account for our results.

Our finding that the impairment of CAR performance caused by barbital was significantly increased by chronic treatment with morphine should be compared with that of Bousquet *et al.* [2] who reported no such increase in sleeping time. Although the actual difference that they observed was in the same direction as ours, it was not statistically significant. Their chronic treatment consisted of four daily IP injections of 20 mg/kg of morphine sulfate and it is possible that a more prolonged and/or severe schedule might have given results more similar to ours. Nevertheless, in both their experiment and ours the effect seen with barbital was less than that seen with the other barbiturates. Therefore, these results imply that changes in drug metabolism caused by chronic treatment with morphine make the major contribution to the observed potentiation of the pharmacological effects of the barbiturates. However, because the responses to all four barbiturates that we tested appeared to be qualitatively similar, these results suggest that dispositional factors alone cannot account for all the effects observed and that other factors also may be involved.

This latter possibility is supported further by our finding that the impairing effects on the CAR of both diazepam and ethanol were also enhanced by chronic treatment with morphine. These two drugs have central depressant properties but are unrelated to the barbiturates in chemical structure and routes of disposition. In neither of these cases could we find evidence that changes in disposition were responsible for the exaggerated behavioral impairment. Indeed, although changes in the disposition of diazepam and ethanol were suggested, they were in a direction that would presumably lead to lessened effect rather than to the potentiation actually observed.

For diazepam we found that the levels of total ^{14}C in plasma, brain, and liver were actually lower in rats treated chronically with morphine than in controls after IP administration of ^{14}C-diazepam in 0.01 N HCl. This effect was not seen 1 or 2 hours after implantation of two morphine pellets [19], which implies that an acute interaction was not responsible. Also, this effect was not seen after IV administration of ^{14}C-diazepam in a propylene glycol vehicle suggesting, perhaps, that chronic treatment with morphine caused changes that led to poorer absorption of ^{14}C-diazepam from the peritoneal cavity. One possibility in this regard is that chronic treatment with morphine caused the low-aqueous-soluble diazepam to precipitate more readily from the acidic vehicle used for IP injection than in controls and thus lessened the amount of drug absorbed. We chose this vehicle for the behavioral experiment to avoid the possibility that the propylene glycol might interact with the diazepam and/or the chronic morphine treatment to confound the behavioral results since depressant effects have been noted with this vehicle at high concentrations [6]. In either case the sedative effect of diazepam was observed to be greater in the rats treated chronically with morphine than in controls and therefore neither the route of administration nor the vehicle used per se are likely to account for the exaggerated behavioral effect.

Levels of toluene-extractable ^{14}C-diazepam after IP administration were also lower in plasma, brain, and liver after chronic treatment with morphine than in controls. TLC indicated that ^{14}C-diazepam was the major radiolabeled component present in the extracts at 1 and 2 hours. At later times several metabolites of diazepam were prominent, especially in the liver. Because some of the metabolites of diazepam are pharmacologically active [22], it is possible that they contributed to the behavioral effects observed. Further experiments with these active metabolites, such as oxazepam, might be expected to clarify this situation. In any event, there was no evidence that the ratios of diazepam to or among its metabolites were altered by chronic treatment with morphine, thus arguing against an alteration in metabolism as an explanatory mechanism.

Ethanol levels in brain were not higher in rats treated chronically with morphine than in controls. Indeed, the levels of ethanol appeared to decrease faster in the morphine-pelleted rats than in the placebo-pelleted rats. This effect was not large and it was significant only in plasma in this experiment. However, we repeated the experiment twice and obtained similar results [19], although their interpretation and relevance to the behavioral results are obscure at this time.

The experiments to determine the effects of chronic treatment with morphine on the disposition of ^{14}C-pentobarbital, ^{14}C-diazepam, and ethanol were all done using untrained rats. This raises a question as to whether the training and the test procedures themselves may have altered the disposition of the test drugs in such a way as to compromise the interpretation of the results obtained on untrained rats. We think that this possibility is unlikely for pentobarbital, at least, because in a preliminary experiment [19] we found that the levels of ^{14}C-pentobarbital in trained, morphine-pelleted rats were also higher than in trained, placebo-pelleted rats after a 60-trial test in which the ^{14}C-pentobarbital was given just before the test. Moreover, the magnitude of the differences was similar to that seen in the untrained rats reported here. Nevertheless, this possibility remains for diazepam and ethanol, especially in view of the fact that the dispositional results obtained from the untrained rats could not be related to the behavioral effects observed in the trained rats.

It is clear from the results of these experiments that the response to a number of drugs with depressant properties can be exaggerated by prior treatment with morphine by our schedule of pellet implantation. We have recently shown that the impairment of the CAR caused by phencyclidine is similarly potentiated [20]. It is also clear that an inhibition of hepatic drug metabolism, which is known to occur in male rats with chronic treatment with morphine, is not sufficient to account for this phenomenon in all cases. What remains unclear at this time is whether the exaggerated responses can be accounted for by an acute interaction between the test drugs and residual levels of morphine. We have provided evidence that this was not the case for pentobarbital. However, similar evidence for the other drugs tested is not currently available. Should such evidence be forthcoming, then by process of elimination at least part of the enhancement may be the result of neurochemical and/or neurophysiological changes in the brain caused by chronic treatment with morphine that make it more sensitive to these drugs. Lesher and Spratto [15] also raised this possibility based on their observation that brain levels of hexobarbital were lower upon awakening from a sedative dose of hexobarbital and required less of the drug to inhibit EEG activity in rats treated chronically with morphine than in controls. Although

more work is needed to verify this hypothesis, such an outcome should not be surprising in view of the known propensity for morphine to induce tolerance and physical dependence, both of which appear to require central mechanisms for their explanation. It will be interesting and, perhaps, revealing to see the extent to which the development of tolerance and physical dependence is related to the postulated increase in drug sensitivity, if it exists. Although not immediately obvious, it may be that all three phenomena depend on similar neurochemical mechanisms for their expression.

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