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A Comparison of the Pharmacology of Two Potent Analgesic Agents, Piminodine[1] (Win 14,098-2) and Win 13,797, with Morphine and Meperidine[2]

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When meperidine (Demerol) was shown to possess marked analgesic properties (Eisleb and Schaumann, 1939) and thus became the first potent synthetic analgesic drug, the importance of the 1,4-substituted piperidine nucleus in providing analgesic activity became apparent. Many modifications of this basic chemical structure have been synthesized and studied pharmacologically in an attempt to achieve greater analgesic potency. Two such derivatives, synthesized by Elpern *et al.* (1959), showing analgesic activity greater than meperidine are piminodine (Win 14,098-2, ethyl 4-phenyl-1-[3-(phenylamino)propyl]piperidine-4-carboxylate) and Win 13,797 (ethyl 4-phenyl-1-[2-(phenylamino)ethyl]piperidine-4-carboxylate).

Pharmacologic studies of these two derivatives in comparison with meperidine and morphine are presented in this report.

METHODS

Unless otherwise indicated, aqueous solutions were administered by the routes indicated and piminodine and Win 13,797 were administered as the ethane sulfonate salts. Morphine and meperidine were injected as the sulfate and hydrochloride salts, respectively.

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Toxicity

The subcutaneous LD_{50} in mice was determined from studies with at least 5 doses and a minimum total number of 100 animals.

Dogs were given doses of 1.3–39 mg/kg intravenously, the highest dosage being lethal. No attempt was made to obtain a precise LD_{50} value for this species.

Nontolerant rhesus monkeys were injected with increasing amounts (no more than one dose in a given animal) of piminodine and Win 13,797 until doses were determined which produced definite depression, but not death. Two animals were injected with each dose of each drug. The psychic depression (stupor) and motor depression (muscular weakness) which resulted from effective doses of these drugs were graded on an 8-grade scale according to the method of Irwin (1954). Respiratory depression produced by the drugs was graded on the scale of mild, intermediate, severe, and very severe.

Analgesia

Two animal experimental methods were utilized for the assay of analgesia. The first was the hot-plate procedure described by Eddy and Leimbach (1953) using male mice. Each drug was administered subcutaneously in at least 5 doses to a minimum total number of 100 animals.

The second procedure for analgesic testing was the Robbins (1955) modification of the hot-wire technique described by Davies *et al.* (1946) for use on rats. At least 70 animals were used for each drug administered subcutaneously in at least 5 doses.

All results were analyzed statistically according to the method of Litchfield and Wilcoxon (1949).

Cardiovascular Actions

Acute vascular tolerance—crossed tolerance to morphine. These experiments were performed on 42 mongrel dogs weighing 8–20 kg and anesthetized with pentobarbital. All injections were made intravenously; blood pressure was recorded from a mercury manometer. Respiratory activity was recorded via a tambour attached to a pneumograph placed low on the thorax. Some animals were allowed to breathe spontaneously and others were respired mechanically.

Perfusion of the hind limb of the dog. The method utilized was that described by Beck (1958). All compounds were initially dissolved in propylene glycol, and then diluted with saline or water as indicated.

Papillary muscle. The papillary muscle preparation of Cattell and Gold (1938), as modified by Bennett (1958), was employed. All muscles were subjected to 2.0 g tension.

Dog heart-lung preparation. A modified heart-lung preparation of Knowlton and Starling (1912) was used. Alteration of cardiac function in this preparation was determined by the Competence Index method of Wollenberger (1947), as well as by the Slope method of Price and Helrich (1955).

Gastrointestinal Action—Isolated Rabbit Jejunal Segment

Rabbits were killed by a blow on the head, and the jejunum was removed promptly, placed in Tyrode's solution, and cut into approximately equal 1-inch segments. Each segment was suspended in Tyrode's solution which was aerated and maintained at a temperature of 37.5° C. Activity was recorded via an isotonic muscle lever and kymograph system.

Neuropharmacologic Studies

Six beagle dogs were prepared with chronically implanted electrodes according to the method of Domino and Ueki (1959). Bipolar stainless steel electrodes were placed epidurally and/or subdurally in various portions of the cerebral cortex including areas 8, 6, 4, 1, 2, 5, and 17. Subcortical electrodes were placed usually in the mesencephalic reticular formation, dorsal or posterior hypothalamus, and portions of the limbic system including the olfactory bulb and medial amygdala. At least 1 month elapsed from the time of the surgical implantation until drug testing. All animals were free of neurologic deficit and had normal electroencephalograms (EEG). A Grass model III 8-channel electroencephalograph was used for electrical recording. The effects of various narcotics were determined in unanesthetized, partially restrained dogs viewed through a window with one-way vision. Thoracic respiration was recorded qualitatively with a rubber bellows connected to a Statham pressure transducer and was recorded on one channel of the electroencephalograph. All sites of electrode placement were verified histologically by the Hess method of iron deposition, staining for the Prussian blue and green color reaction, and counterstaining with thionin.

The effects of 1 mg/kg of piminodine, Win 13,797, and morphine sulfate given intravenously were compared in six dogs. Each agent was administered randomly in a crossover design to the various animals. The

dogs received only one narcotic per week. After the peak of drug effect, nalorphine hydrochloride was given intravenously in a dose of 1 mg/kg.

Tolerance and Addiction

Monkey. (*a*). *Single-dose suppression.* The drugs were evaluated to determine if they had the capacity specifically to suppress morphine abstinence signs. Monkeys which were physically dependent on morphine sulfate (3 mg/kg subcutaneously every 6 hours) were withdrawn until abstinence signs of intermediate severity were present (12–14 hours). Placebo (saline); morphine sulfate, 3 mg/kg; piminodine, 0.1, 0.2, and 0.4 mg/kg; and Win 13,797, 0.05, 0.1, and 0.2 mg/kg, were each administered to 5 randomly selected monkeys. An observer who was unaware of the nature of the treatments established an effect-duration curve for each monkey by grading abstinence signs and/or drug depression below normal excitability by the methods of Irwin (1954). The monkeys were graded just prior to injection of the test drug and at intervals of ½, 1, 2, 3, 4, etc., hours after injection until each monkey returned to its preinjection level of excitability.

(*b*). *Primary physical dependence and tolerance.* Win 13,797 was administered chronically every 6 hours to a group of 3 monkeys to determine whether the drug would produce physical dependence directly. The monkeys had not previously received any narcotic drugs. The dose was increased as tolerance development to the general depressant and anorexic effects permitted. The drug was administered according to the following dosage schedule: days 1–7 (0.1 mg/kg); 8–20 (0.2 mg/kg); 21–29 (0.4 mg/kg); 30–62 (0.8 mg/kg); 63–98 (1.2 mg/kg); 99–109 (2.0 mg/kg); and 110–146 (3.0 mg/kg).

Dog—Primary Physical Dependence

Primary physical dependence studies with piminodine, Win 13,797, and morphine were also conducted in the dogs. Although the dog is ordinarily less well suited to such studies than the monkey, it appears to resemble man more closely than does the monkey with respect to its relative sensitivity to synthetic piperidine derivatives. Each drug was administered subcutaneously every 6 hours to a group of 5 dogs in the following doses: for piminodine, days 1–4 (1.0 mg/kg), 5–26 (2 mg/kg), 27–45 (4.0 mg/kg), and 46–49 (6.0 mg/kg); for morphine sulfate and Win 13,797, days 1–26 (1.0 mg/kg), 27–45 (2.0 mg/kg), and 46–49 (3.0 mg/kg). Each increase in dosage produced anorexia which resulted

in loss of body weight. The dosage was increased after tolerance to the anorexia had developed and lost body weight had been regained.

Withdrawals were precipitated on the 26th, 45th, and 65th days by the subcutaneous administration of 2 mg/kg of nalorphine, and all drugs were abruptly withdrawn on the 69th day.

Distribution and Excretion in Dogs and Monkeys

Specific procedures were developed for the microestimation of piminodine and Win 13,797 to levels of 0.1 μg/ml of plasma or urine or 0.5 μg/g of tissue by a modification of the methyl orange technique of Brodie and Udenfriend (1945). These procedures were shown to be specific by comparison of known drugs and unknown drugs extracted from urine and compared in buffer distribution studies.

Estimation of piminodine. To a 35-ml glass-stoppered, centrifuge tube were added successively (a) 5 ml water solution of drug, or urine, or plasma, or tissue homogenate; (b) 15 ml (purified as described by Woods *et al.*, 1951) ethylene dichloride ($EtCl_2$); and (c) 3 ml of 0.5 *M*, pH 7.5, phosphate buffer. The sample was shaken 30 minutes in an International Shaker Machine at a frequency of about 280 oscillations per minute in tube holders as described previously (Woods *et al.*, 1951) and was centrifuged 4 minutes; the aqueous (upper) layer was then removed with a clean glass tip connected with a water aspirator. The $EtCl_2$ was shaken twice with 5 ml of 0.2 *N* NaOH and once with 5 ml of 0.25 *M*, pH 5.1, phosphate buffer. Each time the mixture was shaken 1 minute by hand, then centrifuged 4 minutes, and the aqueous (upper) layer was removed as above. An aliquot of 13 ml of the $EtCl_2$ layer was transferred to a clean centrifuge tube, 5 ml of 0.5 *N* HCl was added, and the mixture was shaken for 10 minutes. The mixture was centrifuged 4 minutes and the $EtCl_2$ (lower) layer was removed with a *clean aspirating tube.* An aliquot of 4.5 ml of the acid solution was transferred to a clean centrifuge tube, 10 ml of pure $EtCl_2$ and 1 ml of 2.5 *N* NaOH were added, and the samples were shaken mechanically for 30 minutes. The mixture was centrifuged 4 minutes, then the water layer was removed. The $EtCl_2$ layer was shaken 1 minute by hand with 5 ml of 1 *M*, pH 8.6, phosphate buffer as noted above and centrifuged 4 minutes; then the aqueous layer (upper) was removed.

Nine milliliters of the $EtCl_2$ was pipetted into a clean centrifuge tube, and 0.5 ml of methyl orange reagent[4] (prepared by dilution of some of

[4] Prepared as described by Woods *et al.* (1953).

the stock solution with an equal volume of 1 *M* boric acid). The mixture was shaken 10 minutes and centrifuged 4 minutes; most of the methyl orange reagent was removed by aspiration *without loss of* $EtCl_2$. As much of the $EtCl_2$ as possible was transferred *without* any aqueous dye solution, into a clean centrifuge tube. To reduce the blank values, the organic solvent was shaken 1 minute with 3 ml of diluted methyl orange reagent (1.6 ml stock solution diluted to 200 ml with 1 *M* boric acid) and centrifuged 4 minutes; the aqueous methyl orange layer was removed by aspiration. Seven milliliters of the above $EtCl_2$ containing the methyl orange complex of the drug was pipetted into a clean centrifuge tube, 1 ml of 2 *N* HCl was added, and the mixture was shaken mechanically for 10 minutes and centrifuged 4 minutes; the $EtCl_2$ layer (lower) removed carefully by aspiration without loss of aqueous solution. Most of the colored acid solution was transferred with a 1-ml pipette to a microcell and read at 515 mμ in the Beckman DU spectrophotometer.

Duplicate determinations on known standards, having a concentration in the range of the unknown samples, were carried through the procedure with each set of unknowns.

The recovery of known amounts of piminodine was about 110 ± 20 (S.D.) % for the range of 0.1–2.0 μg/ml added to plasma or urine, or above 0.5 μg/g added to tissue.

Estimation of Win 13,797. To a 35-ml glass-stoppered centrifuge tube were added successively (a) 5 ml of water solution of drug, plasma, urine, or tissue homogenate; (b) 15 ml of $EtCl_2$; and (c) 3 ml of 0.5 *M* pH 7.5, phosphate buffer. The same was shaken mechanically for 30 minutes at a rate of 280 oscillations per minute. The mixture was then centrifuged for 4 minutes as described previously, and the aqueous layer (upper) was removed by aspiration. The $EtCl_2$ was washed once by shaking 1 minute by hand with 5 ml of the following: twice with 0.2 *N* NaOH, once with 0.025 *N*, pH 5.1, phosphate buffer, and once with 0.1 *N* HCl.[5] The manipulations of centrifugation and aspiration of the aqueous layer were performed after each wash. The $EtCl_2$ was shaken mechanically with 5 ml of 0.1 *N* HCl for 10 minutes and centrifuged; the acid layer was removed by aspiration. An aliquot of 13 ml of the $EtCl_2$ was transferred to a clean centrifuge tube and shaken with the methyl orange reagent as described for piminodine except that 10 ml of the $EtCl_2$ was transferred for the final extraction of the dye into 1 ml of 2.0 *N* HCl.

[5] Under these conditions the amine is not extracted into the aqueous acid phase, thus indicating the very weak basic nature of the Win 13,797.

The recovery of known amounts of Win 13,797 was about 108 ± 18 (S.D.) % for the range of 0.1–2.0 μg/ml added to plasma or urine, or above 0.5 μg/g added to tissue.

Preparation of tissues. Three grams of tissue (it may be necessary to mince some tissues before they can be homogenized easily) was homogenized in 12.0 ml of 0.5 *M*, pH 7.5, phosphate buffer with the glass tube cooled in an ice bath. Two 5-ml portions of the homogenate were pipetted into two clean centrifuge tubes and analyzed in duplicate according to the procedures noted above.

The following tissues and body fluid were analyzed: brain, lungs, kidney, liver, spleen, skeletal muscle, cardiac muscle, fat (omental), low duodenum, and gall bladder bile.

RESULTS AND DISCUSSION

Toxicity

The LD_{50} values of piminodine and Win 13,797 in mice in comparison with morphine are given in Table 1. The animals usually died after convulsions.

TABLE 1

COMPARISON OF LETHAL AND ANALGESIC POTENCY OF PIMINODINE, WIN 13,797, MORPHINE AFTER SUBCUTANEOUS INJECTION INTO MICE

Drug	LD_{50}, mg/kg (19/20 confidence limits)	AD_{50}, mg/kg[a] (19/20 confidence limits)
Piminodine	228 (218–234)	1.34 (0.99–1.81)
Win 13,797	175 (165–181)	0.86 (0.78–0.95)
Morphine	412 (301–561)	4.4 (3.9 –4.9)

[a] These values were obtained with the hot-plate method for testing analgesia.

The intravenous administration of piminodine or Win 13,797 to dogs (total of 8 dogs and 11 dogs, respectively, for each drug) gave the following results:

1. After doses of 1.3, 3.2, and 6.5 mg/kg, neither drug produced immediate excitement and disorientation as observed after the intravenous injection of comparable doses of morphine. However, typical sedation, narcosis, and the hyenoid gait resulted, the latter being less pronounced than that seen after morphine. In identical dosage, Win 13,797 produced more profound narcosis lasting for a longer period than that observed with piminodine. The intravenous injection of nalorphine hydrochloride

(3 mg/kg) immediately antagonized the depressant effects of both drugs. Salivation occurred after the administration of each drug, but emesis was not produced in any animal by either drug.

2. After doses of 13 mg/kg, both drugs produced tremors or mild convulsions within a very few minutes. The stimulation was followed by a long period of depression, but with survival of the animals.

3. After doses of 26, 32, and 39 mg/kg (1 dog after each drug at each dosage), both drugs produced almost immediate convulsions, principally of the grand mal type. The dogs survived from doses of 26 and 32 mg/kg but died after 39 mg/kg. The one dog given the latter dose of Win 13,797 died in 30 minutes, and that given piminodine died in 5 minutes. It was quite evident that with the higher doses (13, 26, 32, and 39 mg/kg) piminodine exhibited a more pronounced stimulant action on the central nervous system than Win 13,797.

The subcutaneous injection of 0.1 mg/kg of piminodine and 0.5 mg/kg of Win 13,797 to nontolerant monkeys produced sedation, slight ataxia, and slight respiratory depression. The administration of double the above doses of each drug resulted in more marked depression (moderate stupor grade 4 depression) of the central nervous system, slight ataxia, and more marked depression of respiration. Thus, Win 13,797 is about twice as potent as piminodine as a depressant of the monkey. The monkey is very sensitive to these agents as compared with the dog or man.

Analgesia

The analgesic potencies of piminodine and Win 13,797 are compared with morphine in the mouse and the rat in Tables 1 and 2. Both piminodine and Win 13,797 are more potent than morphine.

TABLE 2

COMPARISON OF THE ANALGESIC POTENCY OF PIMINODINE, WIN 13,797, AND MORPHINE AFTER SUBCUTANEOUS INJECTION IN RATS AS MEASURED BY THE HOT-WIRE TAIL-FLICK METHOD

Drug	Piminodine	Win 13,797	Morphine
AD_{50}, mg/kg (19/21 confidence limits)	0.84 (0.65–1.2)	0.13 (0.08–0.21)	1.3 (0.89–2.1)
Latent period	10 Minutes	10 Minutes	10–30 Minutes
Duration	45–60 Minutes	30–45 Minutes	60 Minutes

Cardiovascular Action

Acute vascular tolerance in pentobarbitalized dogs—crossed tolerance to morphine. (a). Spontaneous respiration. The initial intravenous injection of 1.3 mg/kg (1.0 mg/kg as free base) uniformly reduced the blood pressure to a moderate level (viz., to about 90–100 mm of mercury)

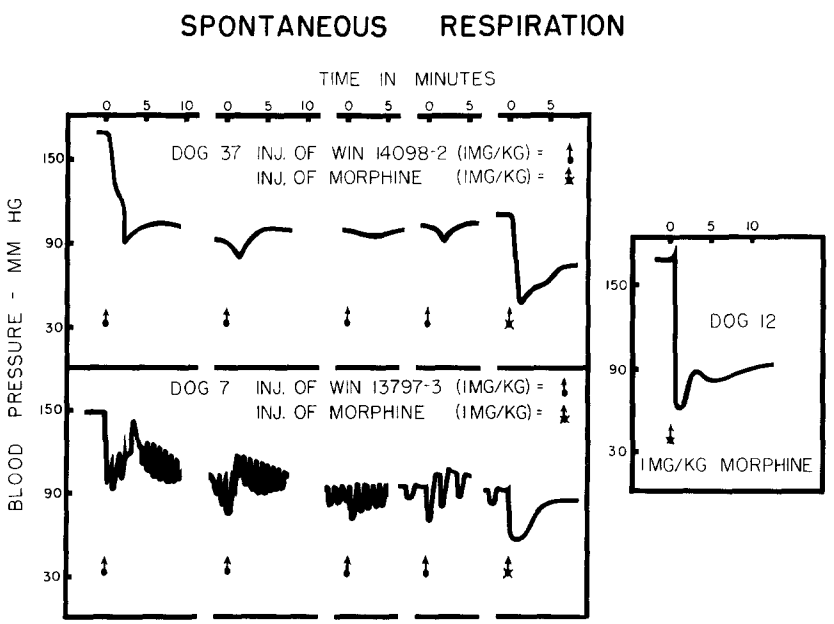

FIG. 1. Typical results after repeated intravenous injection of 1.3 mg/kg of piminodine and Win 13,797 (the 1.0-mg/kg values are free base) followed by 1.3 mg/kg of morphine sulfate (1.0 mg/kg as free base) as compared with the effect of a single injection (no prior treatment with narcotic agent) of morphine (block on the right). All animals respired spontaneously; the interval between injections was 15 minutes. Time in minutes is plotted along the abscissa, and blood pressure in millimeters of mercury along the ordinate.

(Fig. 1) which persisted throughout the experiment. Subsequent injections at 15-minute intervals of 1.3 mg/kg (1.0 mg/kg as free base) of either piminodine or Win 13,797 did not result in an additional significant reduction in blood pressure. Morphine sulfate in identical initial dosage (no drug having been administered previously) produced a more profound hypotension (Fig. 1).

In some animals the intravenous injection of 2.6 mg/kg (2 mg/kg as

free base) of either drug produced respiratory depression followed in 2–3 minutes by vascular collapse and death in approximately one-half of the animals. The intravenous administration of 5.2 mg/kg (4 mg/kg as free base) of either drug produced the above respiratory depression followed by death in nearly every animal (Fig. 2B). The intravenous administration of nalorphine hydrochloride (3 mg/kg) antagonized these respiratory depressant effects of piminodine and Win 13,797.

The administration of three or four consecutive doses of 1.3 mg/kg (1.0 mg/kg as free base) of either drug by the intravenous route showed partial or complete vascular tolerance to this dose. Such animals showed a reduced hypotensive effect (crossed tolerance) of 1.3 mg/kg (1.0 mg/kg as free base) of morphine administered intravenously.

(*b*). *Artificial respiration.* Since the above results indicated that death with doses of 2.6–5.2 mg/kg of piminodine and Win 13,797 was primarily due to respiratory depression, the effect of artificial respiration was studied. Doses of 5.2 mg/kg of either drug administered intravenously (i.v.) to dogs anesthetized with pentobarbital and respired mechanically produced hypotension more pronounced than that following the administration of doses of 1.3 mg/kg, but generally less than that produced by 1.3 mg/kg of morphine sulfate. In such animals the repeated administration of the large doses (5.2 mg/kg; 4 mg/kg as free base) demonstrated the development of acute vascular tolerance and partial cross tolerance to morphine (Fig. 2). Cardiovascular function remained adequate as long as sufficient respiratory exchange was provided.

Dog hind-limb perfusion. Piminodine and Win 13,797 were equipotent in producing vasodilation in the femoral vascular bed of the dog, whether administered i.a. or i.v. A total dose of 0.1 mg of either compound, injected i.a., caused a 30% decrease in limb peripheral resistance which returned to control values within 2 minutes. A dose of 0.5 mg/kg of either compound, injected i.v., produced a slight transient fall in mean systemic blood pressure, not more than 10 mm Hg, which was associated with an initial slight transient reflex increase in limb perfusion pressure. After this, as the drug reached the hind limb after passage through the pump system, a 30–40% decrease in limb perfusion pressure was observed which returned to normal within 15 minutes.

Studies employing this technique would suggest that there appears to be no acute centrally mediated vasodilatation with the doses employed in this part of the study. The hypotensive effect of piminodine and

Win 13,797 can be explained, in part at least, by direct peripheral vasodilatation of vascular areas similar to the femoral bed.

Papillary muscle and dog heart-lung preparations. Approximately a 10% depression of contractility of the eudynamic cat papillary muscle was noted after 30 minutes' exposure to a concentration of 1.0 mg/l

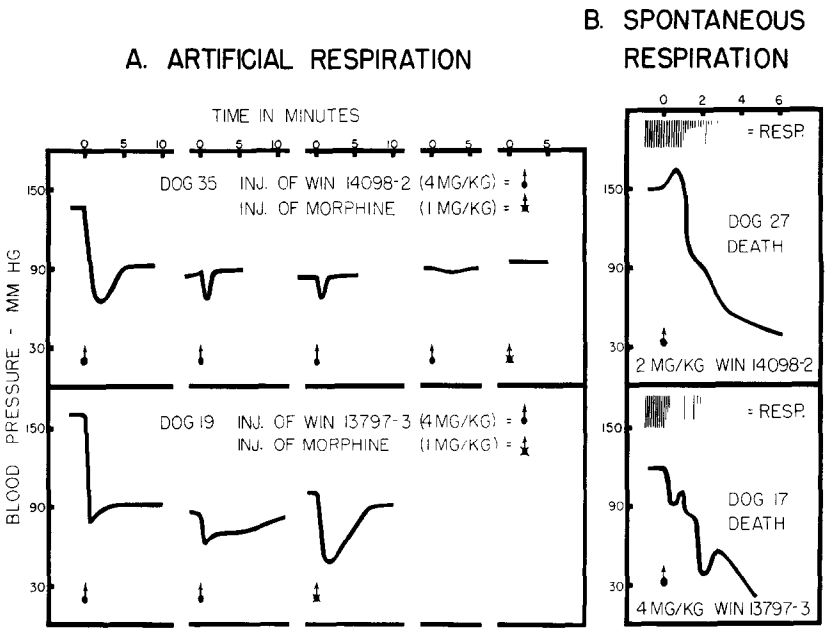

FIG. 2. Comparison of the effect of the intravenous injection of 2.6–5.2 mg/kg (2–4 mg/kg as free base) of piminodine and Win 13,797 in dogs on artificial respiration (A) and dogs respiring spontaneously (B). Where applicable the interval between injections was 15 minutes. Time in minutes is plotted along the abscissa and blood pressure in millimeters of mercury along the ordinate.

piminodine in Ringer's solution, whereas approximately 50% depression was obtained after 30 minutes' exposure to a concentration of 10 mg/l. This depression could not be attributed to pH changes of the bathing solution as a result of drug administration. No potentiation of barbiturate-induced myocardial depression was observed with either agent. The depression produced by these agents at 10 mg/l, unlike an equal degree of depression induced by pentobarbital, was not reversed within 1 hour by replacing the bath solution with fresh Ringer's solution. No spon-

taneity, missed beats, or contracture was evident even with severe myocardial depressant concentrations.

Piminodine and Win 13,797 are myocardial depressants in the dog heart-lung perparation when sufficient drug is administered (Table 3). Slight depression at 100 μg/l, and severe depression at 1 mg/l, was observed for both compounds, employing the Slope method to determine

TABLE 3

COMPARISON OF THE DEPRESSANT EFFECTS OF PIMINODINE, WIN 13,797, AND MEPERIDINE ON UNFAILED DOG HEART-LUNG PREPARATIONS

Drug (μg per liter of circulating blood)	Competence index[a]			Slope[b]		
	Prep. no. 1 Piminodine (%)	Prep. no. 2 Win 13,797 (%)	Prep. no. 3 Meperidine (%)	Prep. no. 1 Piminodine (%)	Prep. no. 2 Win 13,797 (%)	Prep. no. 3 Meperidine (%)
Control	100	100	100	100	100	100
10[c]	98	100	100	86	92	94
100	96	100	98	72	86	91
1,000	92	100	98	41	49	74
10,000	22	46	98	12	16	44
20,000	0	0	81	<1	<1	17

[a] $CI = \frac{H - RAP}{H} \times 100.$

[b] $S = \frac{S\ control - S\ drug}{S\ control} \times 100$ where S control = stroke volume/RAP during control and S drug = stroke volume/RAP after drug administration.

[c] Ten micrograms of drug was added after control period. Effect was determined after a 30-minute exposure. Concentration was then increased to 100 μg and effect determined after an additional 30-minute exposure. Concentration then increased to 1000 μg, etc. All drug solutions were prepared in 10% propylene glycol and saline.

cardiac function. If cardiac function is determined by the Competence Index method, the same qualitative results apply; however, severe myocardial depression is not noted until concentrations of 10 mg/l are attained. As was noted in the cat papillary muscle, piminodine is more of a depressant than Win 13,797, but again both compounds were more depressant than meperidine on an equimolar basis. As expected from initial results in the papillary muscle preparations, nalorphine, 2 mg/l, was ineffective in antagonizing the cardiac depression produced in the heart-lung preparation by the two agents at 10 mg/l. However, ouabain, 100

μg/l, was quite effective in reversing the depression produced by either piminodine or Win 13,797 at 10 mg/l.

Piminodine is somewhat more a cardiac depressant than Win 13,797. This difference cannot be explained on a greater penetration of piminodine into cardiac muscle in light of distribution studies carried out in this study. In any event, the doses of either agent required for moderate cardiac depression are much greater than doses necessary to obtain central nervous system activity. Both compounds were more potent than meperidine as cardiac depressants on an equimolar basis. However, the ratio of analgetic activity to cardiac depressant activity of these compounds compared to meperidine would appear to be approximately the same.

Gastrointestinal Actions—Isolated Rabbit Jejunal Segment

Piminodine, Win 13,797, and meperidine were studied with respect to a comparison of their effects on the isolated rabbit jejunal segment. No effect was observed with any of the three compounds at concentrations of 10 μg per liter of Tyrode's solution. Approximately an 80% decrease in the average amplitude of contraction was observed after a 30-minute exposure to 1 mg/l piminodine in Tyrode's solution. At 5 mg/l, either piminodine or Win 13,797 caused an almost complete suppression of amplitude of contraction after a 10-minute exposure whereas meperidine produced a 60–70% depression of amplitude at this concentration after a 30-minute exposure. Thus, Win 13,797 was less effective than piminodine, but both these compounds were more effective than meperidine, which parallels the results obtained with cardiac muscle. The frequency of contractions was not altered by any of these three agents, even when the average amplitude of contraction was markedly depressed. In addition, all three agents caused an equal degree of relaxation of tonus. Nalorphine, 2 mg/l of Tyrode's solution, was ineffective in reversing the depression due to 5 mg/l of piminodine or Win 13,797.

Thus, the actions of piminodine, Win 13,797, on isolated jejunal segments were qualitatively the same as those obtained with meperidine, viz., a decrease in amplitude of contraction and increase in resting length of the strip. Such activity would not be anticipated with doses required for desired central nervous system activity.

EEG Effects in Dogs with Chronically Implanted Electrodes

In general, the gross behavioral and EEG phenomena following the intravenous administration of piminodine, Win 13,797, and morphine

paralleled one another. After drug injection the animals appeared less agitated in the testing apparatus and showed motor weakness, especially of the hindquarters. The animals frequently had to be supported in the recording apparatus. The well-known signs of morphinelike action were present, including dulling of awareness, marked salivation, and defecation. These effects became maximal in approximately 15–30 minutes after the injection of each drug. At the height of the actions of morphine the EEG showed generalized high voltage slow waves in both the cortical and subcortical structures. However, EEG arousal with peripheral afferent stimulation was easily obtained. Electrical activity in the olfactory bulb was decreased paralleling a decrease in the depth of respiration. The administration of 1 mg/kg of nalorphine promptly reversed the gross behavioral and the EEG effects of morphine. Characteristically the electrical activity of the olfactory bulb and amygdala were very much enhanced after the administration of nalorphine to the narcotized dogs. The administration of nalorphine alone to normal dogs in comparable doses did not produce such enhanced activity.

The actions of piminodine were qualitatively similar but definitely much weaker than morphine, both behaviorally and electroencephalographically. The EEG effects of this agent are illustrated in Fig. 3. The control activity for the various brain areas is fairly typical, as can be observed in panel A. In the neocortical areas as well as in the dorsal hypothalamus and mesencephalic reticular formation generally low voltage, fast frequency activity was seen. The activity of the olfactory bulb and medial amygdala consisted of periodic bursts of high frequency waves that were generally synchronous with respiration. Blockade of the ipsilateral nostril prevented this burst activity from appearing in both the amygdala and the olfactory bulb. After administration of 1 mg/kg of piminodine some slow waves were observed in both the neocortical areas. Respiratory depth was not particularly affected. Frequently panting was noted and some olfactory bulb activity was still present as illustrated in panel B, Fig. 3. As in the case of morphine, the injection of nalorphine promptly restored the low voltage, fast frequency cortical EEG activity to normal while the discharges in the amygdala were enhanced (see panel C, Fig. 3).

The actions of Win 13,797 were qualitatively and quantitatively similar to those of morphine.

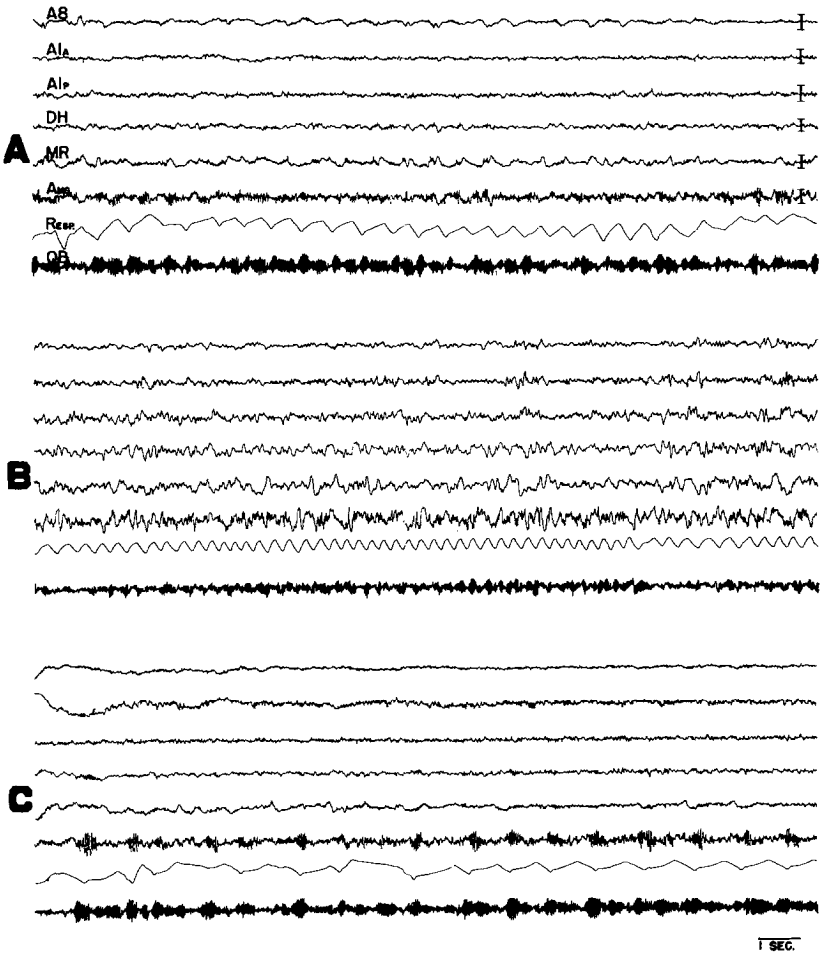

FIG. 3. EEG effects of piminodine. Bipolar recordings were taken throughout: A8, cortical area 8; A1a, anterior portion of cortical area 1; A1p, posterior portion of cortical area 1; DH, dorsal hypothalamus; MR, mesencephalic reticular formation; Amg, medial amygdala; Resp, thoracic respiration; inspiration is down, expiration is up; OB, olfactory bulb. Panel A, control. Panel B, 15 minutes after the intravenous administration of 1 mg/kg of piminodine. Panel C, 1 minute after the intravenous administration of 1 mg/kg of nalorphine hydrochloride.

Tolerance and Addiction

Monkey. (*a*) *Single-dose suppression.* Figure 4 represents the results of the single-dose suppression studies with placebo, 3 mg/kg morphine sulfate, 0.1 mg/kg piminodine, and 0.05 mg/kg Win 13,797. Each point on each curve represents the average grade of the group of 5 monkeys. Taking into account the difference in the preinjection grades, each of the three drugs suppressed morphine abstinence signs to approximately the

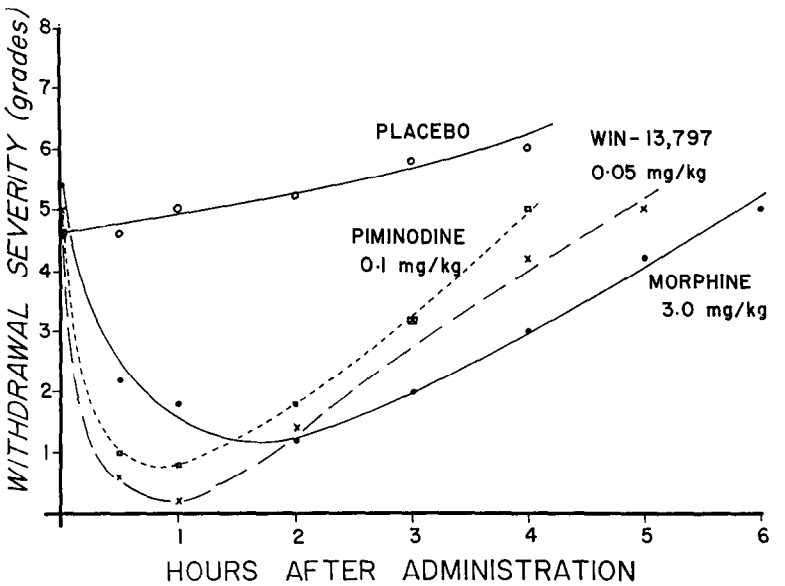

FIG. 4. Effect of piminodine and Win 13,797 on single-dose suppression studies in monkeys showing abstinence signs of intermediate intensity.

same degree. The duration of action of piminodine was less than that of Win 13,797, which was less than that of morphine (duration measured as the time required for the monkeys to return to the preinjection level of excitability). The results of other doses tested by this technique are summarized in Table 4.

The single-dose suppression studies indicated that both piminodine and Win 13,797 possess morphinelike physical dependence capacity in the rhesus monkey. The former is approximately 30 times as potent as morphine in this respect, and the latter about 60 times as potent as morphine. The effects of single doses of these drugs in nontolerant mon-

keys confirmed this high order of potency of these drugs relative to morphine in this species.[6]

(*b*) *Primary physical dependence to Win 13,797.* Stupor of intermediate (grade 4) degree was produced by the initial dose (0.1 mg/kg) of Win 13,797, but no cumulative effects resulted upon repeated administration. After each increment in dosage, effects at least as intense as those of the initial dose reappeared. The only evidence of tolerance development to 3 mg/kg was a slight decrease in the duration of action.

TABLE 4

SUMMARY OF SINGLE-DOSE SUPPRESSION STUDIES IN THE MONKEY AFTER SUBCUTANEOUS INJECTION OF PLACEBO, PIMINODINE, WIN 13,797, AND MORPHINE

Drug	Dose (mg/kg)	Maximum suppression (grades)	Time of peak effect (hours)	Duration of action (hours)
NaCl	4.5[a]	—	—	—
Morphine sulfate	3.0[a]	4.2	2	6
Piminodine	0.1[a]	3.8	1	3.7
	0.2	4.2	½ and 1	4.7
	0.4	7.6	½ and 1	6
Win 13,797	0.05[a]	4.8	1	5
	0.1	6.0	½	5
	0.2	6.8	½	5.7

[a] These data are graphically represented in Fig. 4.

No anorexia resulted when the dose was raised to 2 and 3 mg/kg. One of the monkeys was found dead on the morning of the 117th day, but the cause of death was not apparent at autopsy.

Severe withdrawal signs (grade 7) appeared in all three monkeys and persisted at peak severity for 3 hours after nalorphine administration on the 36th day. A 36-hour abrupt withdrawal was conducted in the 125th

[6] These data were obtained by a double-blind technique in a program of evaluating physical dependence liability sponsored by the Committee on Drug Addiction and Narcotics of the National Research Council. Dr. N. B. Eddy, Secretary, supplied the drugs among a series of coded unknowns. The data were confirmed in subsequent repetition of the experiments in which the identities of the compounds were known.

day to determine the rate of onset of abstinence signs. Signs began to appear at the 7th hour of withdrawal, but the intensification was relatively slow. At the 36th hour signs were of mild intensity (grade 2) in one monkey and intermediate (grade 4) in the other. Chronic treatment with 3 mg/kg Win 13,797 was then reinstituted, and neither monkey showed any evidence of loss of tolerance over the 36-hour period of withdrawal.

Both monkeys showed mild gastrointestinal signs, severe dyspnea, severe muscular weakness, and prostration within 5 minutes of the injection of nalorphine on the 146th day of treatment. Ten minutes after injection both monkeys had brief (30–60 seconds) tonic seizures. Each monkey had several seizures over a 15-minute period after which it lay quietly. Muscular weakness was of such severity that the monkeys could not maintain the sitting posture. One and one half hours after injection of nalorphine, muscular strength began to return and extreme motor restlessness persisted for ½ hour. The two monkeys began to fight and this precipitated a prolonged seizure in one animal which expired before the seizure could be antidoted. The second monkey then began to scream and to circle, collapsed, and died of respiratory failure. The primary physical dependence study with Win 13,797 confirmed the fact that this agent is very potent in the monkey and that it possesses morphinelike physical dependence capacity.

Dog—primary physical dependence. Initial doses of morphine, piminodine, and Win 13,797 produced salivation, retching, defecation, and sedation in all dogs. Vomiting was produced only in those dogs which received Win 13,797, and cumulative depression was observed only with this drug. Tolerance to the sedative effects developed within 2–6 days, but tolerance development to the gastrointestinal effects was minimal.

Narcotic abstinence signs observed in dogs included nausea, retching, vomiting, diarrhea, coughing, piloerection, shivering, hyperpnea, dyspnea, restlessness, growling, fighting, howling, apprehension toward the observer, muscular tenderness, muscular tremors, "digging," and signs of sexual excitation. The withdrawal intensities are summarized in Table 5.

Compared to morphine, the piminodine withdrawals were characterized by milder muscular signs and more severe gastrointestinal signs. Sexual signs, gastrointestinal signs, and muscular tremors were the most prominent features of withdrawal from Win 13,797.

These studies in the dog also demonstrated that piminodine and Win 13,797 produce morphinelike effects in nontolerant animals and have the

capacity to induce morphinelike physical dependence. The dog, however, is relatively much less sensitive to these compounds than the monkey. On the basis of acute effects in nontolerant dogs and the intensities of the abstinence syndromes in physically dependent dogs the relative potencies of the drugs studied were: morphine sulfate, 1.0; Win 13,797, 1.0; piminodine, 0.5. The relative sensitivity of the dog to these compounds thus parallels that of the rat and man. The reason why the monkey should be so sensitive to these particular piperidine derivatives remains obscure. While this quantitative discrepancy serves to caution against the direct extrapolation of data regarding narcotic analgesics from monkey to man, it does not affect the superior usefulness of the monkey for the qualitative evaluation of this class of drugs.

TABLE 5

SUMMARY OF PRIMARY PHYSICAL DEPENDENCE STUDIES IN DOGS

Drug	Day of treatment	Dose (mg/kg q6h)	Type of withdrawal	Average maximum withdrawal intensity (grade)
Morphine sulfate	26	1.0	Nalorphine-induced	3.0
	45	2.0	Nalorphine-induced	3.8
	65	3.0	Nalorphine-induced	4.3
	69	3.0	Abrupt	3.0[a]
Piminodine	26	2.0	Nalorphine-induced	2.8
	45	4.0	Nalorphine-induced	4.8
	65	6.0	Nalorphine-induced	5.8
	69	6.0	Abrupt	3.0[b]
Win 13,797	26	1.0	Nalorphine-induced	2.3
	45	2.0	Nalorphine-induced	3.7
	65	3.0	Nalorphine-induced	4.8
	69	3.0	Abrupt	3.0[c]

[a] Onset of signs was at 8 hours, peak intensity at 30 hours; duration of withdrawal was 8 days.

[b] Onset of signs was at 10 hours, peak intensity 12 through 72 hours; duration of withdrawal was 10 days.

[c] Onset of signs was at 10 hours, peak intensity 36 through 60 hours; duration of withdrawal was 9 days.

Physiological Disposition

Plasma levels and urinary excretion. Two female mongrel dogs were injected subcutaneously with a dose of 6.5 mg/kg for each drug, piminodine and Win 13,797. The concentration in plasma fell within the area of lowest sensitivity of the method, and accordingly, the values were somewhat erratic. Levels never exceed 0.3 μ (free base) per milliliter of plasma, and generally the maximum value occurred 60–90 minutes after administration. The two dogs injected with piminodine eliminated in the urine 1.4 and 2.4% of the administered dose, respectively. Both dogs receiving Win 13,797 excreted less than 1% of the administered dose.

It is obvious from these data on plasma levels and urinary excretion that the major portion of each of these drugs is metabolized, probably by hydrolysis of the ester configuration.

TABLE 6

CONCENTRATION[a] OF PIMINODINE AND WIN 13,797 IN NONTOLERANT AND TOLERANT DOGS 90 MINUTES AFTER THE SUBCUTANEOUS INJECTION OF 6.5 MG/KG OF DRUG

	Nontolerant		Tolerant	
Tissue or fluid[b]	Piminodine[c]	Win 13,797-3[c]	Piminodine	Win 13,797[c]
Bile	13, 13	11, 9	17	7.7, 9.2
Liver	8.9, 8.4	6.6, 6.1	8.2	—, 2.2
Spleen	7.3, 7.9	6.3, 5	—	—, —
Brain	5.5, 5.2	5.5, 5.4	5	—, —
Kidney	5.8, 5.4	5, 5	5	8.9, 5
Lungs	5, 5	5, 5	6.2	5, 5.1
Cardiac muscle	5.5, 5.4	5.7, 5	6.9	5.4, 6.9
Skeletal muscle	5.5, 5.4	5, 5	5	5, 5
Plasma	Trace	Trace, 0.2	—	—, —

[a] Concentration in micrograms (free base) per milliliter or gram.

[b] Duodenum and omental fat were analyzed for the respective drug in each of the animals. The drug levels were less than 5 μg/g except for the value of 9.3 μg/g for Win 13,797 for one tolerant dog.

[c] Each figure represents one animal and is the mean value of duplicate determinations performed on the tissue or fluid removed from one dog.

Tissue distribution in nontolerant and tolerant dogs. These data are summarized in Table 6. In the nontolerant dog the highest concentrations are found in bile. The concentration of both drugs in brain after the administration of 6.5 mg/kg is about 5 μg (free base) per gram. Fat (omental) was very low 90 minutes after administration of the drug, sug-

gesting minimal solubility of these drugs in neutral fat. In general there was no significant difference between the nontolerant and tolerant animals.

SUMMARY

The toxic and lethal effects of piminodine and Win 13,797 were studied in mice, dogs, and monkeys. The analgesic actions of both drugs were tested in mice and rats and were found to be more potent than morphine.

Vascular studies on piminodine and Win 13,797 have shown peripheral vasodilation and acute vascular tolerance to the hypotensive effect in dogs with partial crossed tolerance to morphine. Dogs anesthetized with pentobarbital were sensitive to the respiratory depressant actions of these drugs. Nalorphine readily antagonizes this respiratory depression.

Piminodine and Win 13,797 are myocardial depressants on the dog heart-lung preparation and the cat papillary muscle, but only in doses or concentrations much higher than would be used therapeutically.

The actions of piminodine and Win 13,797 on the isolated rabbit jejunal segment are minimal.

The qualitative actions of piminodine and Win 13,797 on EEG effects in dogs with chronically implanted electrodes were like those of morphine. Quantitatively, Win 13,797 was equipotent to morphine, and piminodine one-half as effective.

Studies on single-dose suppression in monkey and primary physical dependence in dogs and monkeys indicate that piminodine and Win 13,797 have high addiction liability at least in these species.

Nearly all the administered piminodine or Win 13,797 is altered chemically when injected into dogs. There is no evidence for marked local tissue deposition for either drug.

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