RESEARCH PAPERS

PIRINITRAMIDE (R 3365), A POTENT ANALGESIC WITH UNUSUAL CHEMICAL STRUCTURE

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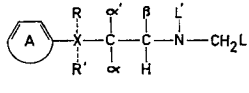
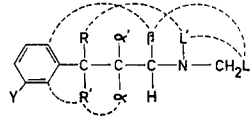
The novelty of the unusual chemical features of the pirinitramide (R 3365) molecule is outlined. It is shown that the compound possesses many morphine-like properties.
In most tests it is more active than morphine by the subcutaneous route, but many times more active orally. As a respiratory depressant agent pirinitramide is possibly less potent in rats than morphine and as an emetic in dogs it is much less active.

MANY synthetic and semi-synthetic organic compounds with pronounced morphine-like activity have been described. All powerful narcotic analgesics have the basic chemical structure (I), in which the nitrogen is usually present as a tertiary, more rarely, secondary or quaternary amine. The basic nitrogen is linked to a flat aromatic ring (phenyl, 2-thienyl) by a chain of three atoms, two of which are carbons and the third X either

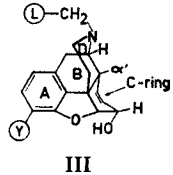


Amines of general structure I.
X = carbon, nitrogen or oxygen.

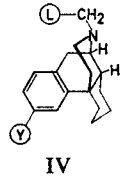


Amines of general structure II.
Y = H, OH, OAc or Oalk;
L′ = H or CH_2L'';
β = H or $CH_2\beta''$;
α = H or $CH_2\alpha''$;
α' = H or OH.

carbon, nitrogen or oxygen. The substituent β on the first carbon atom of this chain is either hydrogen or alkyl. The substituents α and α' on the second carbon atom are two hydrogen atoms, hydrogen and alkyl, or hydroxy and alkyl. The "central atom" X, when carbon or nitrogen, is fully substituted. The substituents L, L′, α, α', β, R and R′ may be



III



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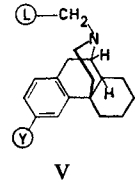
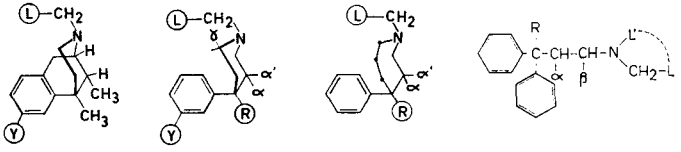


V

III. (−)-Morphine (Y = OH; L = α' = H; absolute configuration).
IV. Morphinans (levorphanol: Y = OH; L = H; absolute configuration).
V. Isomorphinans.

linked with each other and also with the *ortho*-position of ring A. Most of the known morphine-like analgesics are derivatives of general structure II (I: X = carbon). The 5-ring structures related to morphine (structure III shows the absolute configuration of the analgesically active *laevo* isomer, (Beckett and Anderson, 1960), the 4-ring structures of the morphinan type (IV), the B/C-*trans*-isomorphinans (V), the benzomorphans (VI) and their CH_3/CH_3-*cis*-isomers, the 2-ring-compounds related to pethidine (VII), the hexamethyleneimine homologues of VII (VIII) as well as a few less important similar compounds with morphine-like properties, are all derived from II by ring closure between R and L′ to form a piperidine or a hexamethyleneimine-nucleus. The analgesic potency of these drugs depends largely on the nature of substituents L, Y and R (Beckett and



VI VII VIII IX

VI. Benzomorphans (−)-phenazocine: Y = OH; L = $CH_2C_6H_5$; absolute configuration.

VII. Compounds related to pethidine.

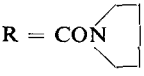
e.g. pethidine: Y = L = α = α′ = γ = H; R = $COOC_2H_5$.
β-prodine: Y = L = α′ = H; α = CH_3; R = $OCOC_2H_5$.
ketobemidone: Y = OH; L = α = α′ = γ = H; R = COC_2H_5.

VIII. Hexamethyleneimine analogues of VII.

IX. 3,3-Diphenylpropylamines

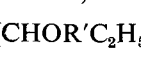
e.g. methadone: R = COC_2H_5; α = H; β = CH_3; NL′CH_2L = $N(CH_3)_2$
isomethadone: R = COC_2H_5; α = CH_3; β = H; NL′CH_2L = $N(CH_3)_2$

phenadoxone: R = COC_2H_5; α = H; β = CH_3; NL′CH_2L = 

dextromoramide: R = CO  ; α = CH_3; β = H; NL′CH_2L = 
(dextroisomer)

Anderson, 1960; Beckett and Casy, 1954; Eddy, 1959; Eddy, Bezendorf and Pellmont, 1958; Eddy, Halbach and Braenden, 1956; Janssen and Eddy, 1960; May and Eddy, 1959; May and Fry, 1957).

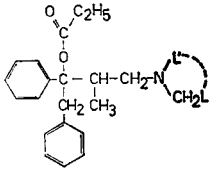
For optimal potency, L must be $CH_2C_6H_5$ or CH_2-2-furyl in III, IV and VI, and $CH_2CHOHC_6H_5$ in VII. In III to VI, Y = OH is optimal, but not necessarily in VII or VIII. In VII or VIII morphine-like activity is associated with R = $COOC_2H_5$, $OCOC_2H_5$, COC_2H_5 or n-C_3H_7 and some lower alkyl analogues of these esters and ketones.

The 3,3-diphenylpropylamines related to methadone (IX) are also derivatives of general structure II (R′ = C_6H_5). High potency is associated with the presence of a methyl-group in α or in β, depending on the nature of substituent R, which may be a ketone (COC_2H_5), a secondary alcohol or its ester ($CHOR'C_2H_5$), a tertiary amide (CO  or $CON(CH_3)_2$)

or a sulphone ($SO_2C_2H_5$). When R is $CONH_2$, CN or OH however, the effects are atropine-like and no analgesic properties are detectable. The potent analgesics of type IX are derivatives of dimethylamine, morpholine, piperidine or hexamethyleneimine. Higher dialkylamines are generally inactive (Janssen, 1959).

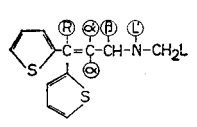
The weakly active propoxyphene-derivatives (X) are chemically closely related to IX.

Isosteric replacement in structure II of phenyl by 2-thienyl leads to the closely related general structure XI, from which the thiambutenes XII are derived. Note the close relationship between IX and XII.

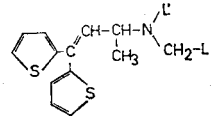


X

Propoxyphene: $NL'CH_2L = NMe_2$



XI



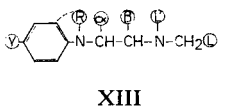
XII

Thiambutenes

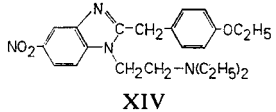
Replacement of carbon in II by nitrogen leads to the derivatives of *N*-phenyl-*N'*-methylethylenediamine of general structure XIII from which the highly potent analgesic 1-diethylaminoethyl-2-(4-ethoxy)-benzyl-5-nitrobenzimidazol (XIV) is derived. In XIV the 5-nitro group as well as the unbranched diethylaminoethyl side chain seem to be important features.

The much weaker phenampromide-like analgesics XV are also derived from structure XIII.

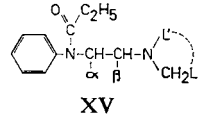
The analgesic activity of a few benzodioxanes of structure XVII which are morphine-like in some respects is evidence for the possibility of



XIII



XIV

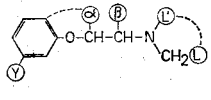


XV

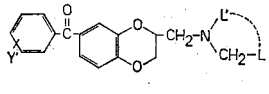
Phenampromide-like analgesics

replacing carbon in II by oxygen to obtain general structure XVI, which is also compatible with morphine-like activity.

A few compounds of structure XVIII were reported to be morphine-like. Their potency is weak and more information is required on their mechanism of action. Whatever the answer to this problem however, there is as yet no exception to the statement that potent morphine-like drugs

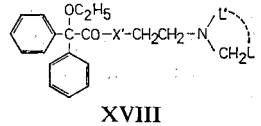


XVI



XVII

Benzodioxanes



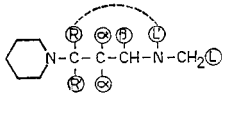
XVIII

X = O or NCH_3

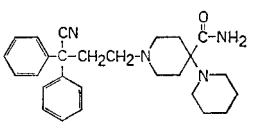
proved in man have a common chemical feature, that is, general structure I in which A is phenyl or 2-thienyl, and X is carbon, nitrogen or oxygen.

In a search for new and potent analgesics, devoid of the classical disadvantages of morphine we have synthesised and screened an extensive series of tertiary amines related to general structure I, but different in at least one respect from the known compounds.

We have found that the chemical variant of I, with the general structure XIX, is compatible with high morphine-like activity. The fact that the piperidino-ring in XIX probably has a chair-conformation shows that the A-ring in I must not necessarily be a flat aromatic structure, as was previously accepted. The presence of this piperidino-ring therefore is

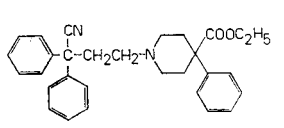


XIX



XX

Pirinitramide* (R 3365)



XXI

Diphenoxylate (R 1132)

regarded as the most important novel chemical feature in the analgesically active compounds of type XIX. In this paper the pharmacology of 2,2-diphenyl-4-(4-piperidino-4-carbamoylpiperidino)butyronitrile, pirinitramide* (R 3365: XX) a typical representative of this new series, will be outlined. The closest known analogues of XX are the analgesically inactive synthetic antidiarrhoeal agents related to diphenoxylate or R 1132 (XXI). (Janssen, Jageneau and Huygens, 1959).

Experimental Methods and Results

We have compared in mice, rats, cats and dogs, the influence of a geometric series of subcutaneous or oral doses (40, 20, 10 . . . mg./kg.) of pirinitramide and of morphine on the characteristic motor activity to various types of stimuli, summarised in Table I.

In tests 1 to 4 of Table I noxious stimuli, that is, stimuli evoking a sensation of pain in man, were used to evaluate appropriate responses in mice, rats and dogs. Contact temperatures of 45° to 60° to the feet not only increase overall motor activity in mice and rats, but also produce other qualitative behavioural changes. In the hot plate test in mice the plate temperature of 55° causes nearly all control mice to lick their paws within 10 sec. after being dropped on the dry plate (Janssen and Jageneau, 1957). In similar conditions Wistar rats react similarly to a dry plate at 55°, but will immediately try to jump out of the restraining glass cylinder when dropped on a plate covered with a thin layer of water. The actual responses depend to a large extent on the plate temperature. On a wet plate at 50° a rat will show considerably increased motor activity without licking or jumping. Pinching a toe of a rat with a forceps results in typical struggle and escape reactions. Tests in which the effects of drugs on the phenomena caused by noxious stimuli are studied, are usually

* Proposed generic name.

referred to as tests for measuring "analgesic" activity. This statement however is valid only for man and not for animals. Pain is a word used for describing a universal subjective experience of mankind. Strictly speaking therefore, pain can be studied only in man. What we are able

TABLE I

SUMMARY OF TESTS FOR STUDYING THE EFFECTS OF DRUGS ON MOTOR ACTIVITY ELICITED BY A VARIETY OF STIMULI, IN MICE, RATS AND DOGS

Species	Test	Situation (unfamiliar unless otherwise noted)	Most important stimuli	Motor activity measured
Mice ..	1. Hot plate test	Dropped on hot plate	Contact heat (55°)	Reaction time of licking reflex (sec.)
Rats ..	2. Cold plate test	Dropped into glass cylinder	New environment	General motor activity (nominal ranking scale)
Rats ..	3. Hot plate test	Dropped on hot plate	Contact heat (50°)	General motor activity (nominal ranking scale)
Rats ..	4. Toe pinching test	Held in hands of observer	Pinching toe of hind paw	Struggling and escape reactions (nominal ranking scale)
Rats ..	5. Open field test	Black arena (open field)	New environment and central illumination	Ambulation, rearing, preening, emotional defecation (ratio scale)
Rats (trained)	6. Weight gain test	Food deprivation schedule	Presentation of food	Food consumption and weight gain in grams per 2 hr.
Rats, dogs (trained)	7. Jumping box test	Familiar avoidance-escape jumping box situation	Buzzer, shock or silence	Pattern of reaction times of avoidance and escape responses (sec.)
Mice (selected)	8. Rotarod test	Balance on rotating rod	Disturbance of balance	Duration of induced coordinating running (sec.)
Mice, rats	9. Righting reflex test	Supine on undulated metal surface (30°)	Release from abnormal position	Reaction time of righting reflex (sec.)
Mice (selected)	10. Fighting test in mice	Isolated aggressive male in familiar cage	Presence of another male	Fighting behaviour (all or none)
Rats ..	11. Palpebral test	Observation cage	Environmental stimuli	Tendency to close or open the eyes (nominal ranking scale)
Rats ..	12. Cornea reflex test	Held in hand of observer	Touching the cornea	Corneal reflex (all or none)
Rats ..	13. Pinna reflex test	Held in hand of observer	Touching the meatus	Pinna reflex (all or none)
Dogs ..	14. Apomorphine test	Special cage	Apomorphine-injection (0·31 mg./kg. s.c.)	Vomiting (all or none)
Rats ..	15. Apomorphine test	Special cage	Apomorphine-injection (1·25 mg./kg. i.v.)	Chewing movements (all or none)
Rats ..	16. Amphetamine test	Special cage	Amphetamine-injection (10 mg./kg. i.v.)	Chewing movements (all or none)
Rats ..	17. Tryptamine test	Special cage	Tryptamine injection (40 mg./kg. i.v.)	Bilateral clonic rythmic movements of front paws (all or none)

to study in animals is not pain itself, but the behaviour of the animal after a stimulus which provokes pain in man (referred to as a "noxious" stimulus in this paper) and also, of course, the influence of a given factor,

such as the administration of an analgesic drug on the reactions of the animal to the noxious stimulus (Janssen, 1959).

Hot Plate Test in Mice (Janssen and Jageneau, 1958)

After subcutaneous injection both pirinitramide and morphine hydrochloride significantly prolong the reaction times of mice. Pirinitramide is faster acting than morphine, while the duration of action of both drugs is similar.

	Pirinitramide	Morphine
mg./kg. s.c.	5·0	10
Onset of action (min.)	8	16
Peak effect (min.)	30	47
Duration of action (min.)	95	92

The slopes of the dose-effect curves do not significantly differ from parallelism and pirinitramide may therefore be said to be almost exactly twice as potent as morphine in this test. A similar investigation of other analgesics gave the following estimated potency ratios, where pirinitramide is 1.

Phenoperidine	=	14	Morphine hydrochloride	=	0·5
Dextromoramide	=	7	Pethidine HCl	=	0·2
Phenazocine	=	4·5	Codeine phosphate	=	0·1
Levorphanol	=	1·5	(+)-Propoxyphene HCl	=	0·07
(±)-Methadone HCl	=	1			

After the injection of ED50-doses, these drugs caused morphine-like excitement, the Straub reaction and mydriasis in nearly all mice.

Cold Plate and Hot Plate Tests in Rats

Method. For each experiment a series of 24 similar pairs of male Wistar rats were selected. The back of one rat of each pair was painted red. Random selection determined the treatment. One rat of each pair was given subcutaneously one of the three doses investigated in each experiment, the other rat being similarly and simultaneously injected with 10 ml. of water per kg. weight. There were eight pairs for each dose and successive pairs were treated at constant intervals of 2 min. The experiment was made by two trained observers, who were unaware of the contents of the solutions as well as of the identity of the treated animal of each pair. Care was taken to rule out conscious or unconscious communication between the observers during the experiment.

One hr. after injection, one of each pair of rats was taken by each of the observers and simultaneously dropped into a restraining glass cylinder onto a copper plate at room temperature and covered with a thin layer of water.

Both rats were observed for 30 sec. The pairs were then dropped in the same order into an identical glass cylinder on a "hot plate" of 50° which was also covered with water, and again observed for 30 sec. At the end of each period both observers independently stated a "preference" for one of the rats of each pair and used a nominal scale to rank the degree of confidence in selection.

This was done according to the following instructions.

Select the animal of each pair that showed less general motor activity than its partner.

Score 1 for failure to observe any difference between both rats which can be considered significant.

Score 2 for a clear difference causing no hesitation in select of the preferred animal.

Score 3 if the observed difference could be due to drug action. The significance of the results is statistically evaluated by binominal analysis, the details of which will be published elsewhere (Janssen, 1961).

Results. The results obtained with a geometric series of subcutaneous doses are graphically summarised in Fig. 1. In these experiments, agreement between the observers was excellent. The conclusion is that

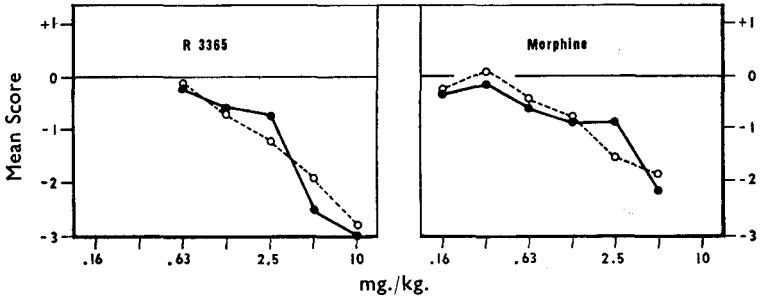


FIG. 1. Hot plate test in rats. ●—● plate at 20° (first trial). o---o plate at 50° (second trial).

pirinitramide and morphine are qualitatively indistinguishable in this test. Both compounds significantly reduce general motor activity as shown by the significantly low frequency of positive scores allocated by the observers. These effects become increasingly pronounced with increasing doses.

We were rather surprised to find that the exploratory motor activity of rats on a cold plate (20°) was inhibited by both compounds to approximately the same extent as the more pronounced motor activity of the same animals on a heated plate of 50°.

The slopes of the "mean score versus dose"—curves of pirinitramide are steeper than the corresponding slopes of morphine Fig. 1, a dose of 0·63 mg./kg. of morphine being slightly but significantly active, whereas the same dose of pirinitramide is inactive. A dose of 5 mg./kg. of pirinitramide however is more active than the same dose of morphine.

The high sensitivity of this simple test procedure is noteworthy as very few pharmacological tests are capable of demonstrating any effect after 0·63 mg./kg. of morphine s.c.

Pinching Test in Rats

Method. The design of the experiment is similar to that used for the hot plate test in rats. Two trained observers select a series of pairs of Wistar rats. They treat one rat of each pair and the other rat as control.

One hour after dosage, they take each pair from a plastic container (one observer) and pinch the toes of the hindpaws with a forceps (the other observer). Repeat as often as desirable.

Without knowing which rat received the drug, they select the animal from each pair which shows less "reactivity" (such as escape or struggling) than its partner. Scores of 1, 2 or 3 are allocated to each selected rat, using the system described for the "cold and hot plate" test. The significance of the results is assessed as before, using binomial statistics.

The "ideal" number of animals per dose may be determined by sequential analysis.

Results. The results obtained after subcutaneous or oral administration of pirinitramide or morphine are graphically summarised in Fig. 2.

After subcutaneous injection pirinitramide is about as active or possibly slightly more active than morphine. After oral administration however

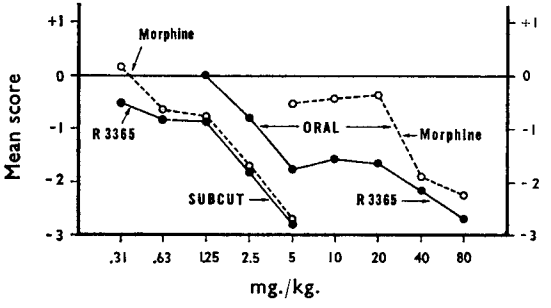


FIG. 2. Mean scores obtained 1 hr. after subcutaneous or oral doses of R 3365 and of morphine in the pinching test in rats (16 pairs per dose).

it is many times more active. A mean score of −2 (clear difference observed in all rats) is obtained after about 2·5 mg./kg. s.c. or 5 mg./kg. orally of pirinitramide and 2·5 mg./kg. s.c. or 40 mg./kg. orally of morphine hydrochloride.

Open Field Test in Rats (Janssen and Jageneau, 1960)

The method consists in assessing ambulation, rearing and emotional defaecation of Wistar rats in an unfamiliar black painted arena (diameter of 1 m.).

Results. The exploratory and emotional behaviour of Wistar rats in an open field situation, measured 1 hr. after subcutaneous injection, is modified in a similar and typical manner by both pirinitramide and morphine (Fig. 3). Motor activity may be either stimulated or depressed, depending on the dose used.

Very high doses (40 mg./kg. s.c. or more) are needed to suppress ambulation, rearing and defaecation completely. At lower doses pirinitramide and morphine cause irregular ambulation-behaviour, periods of increased running activity alternating with periods of complete rest. The mean ambulation scores however are hardly influenced. Increased rearing scores are typical for small or average doses of morphine-like

substances. After injection of 0·63 mg./kg. of pirinitramide and 1·25 mg./kg. of morphine a 100 per cent increase of rearing behaviour was observed. Emotional defaecation decreases with increasing dose levels, morphine being about twice as potent as pirinitramide in this respect.

Qualitatively similar effects are observed with all other morphine-like analgesics in this test, whereas neuroleptics related to chlorpromazine are either ineffective or decrease ambulation, rearing and defaecation, without ever stimulating motor activity.

Weight Gain Test in Rats

Method. Female Wistar rats of about 250 g. in individual metal cages are put on a 22 hr. food deprivation schedule, standard pellets being offered to them *ad libitum* between 10 and 12 a.m. or between 2 and 4 p.m.

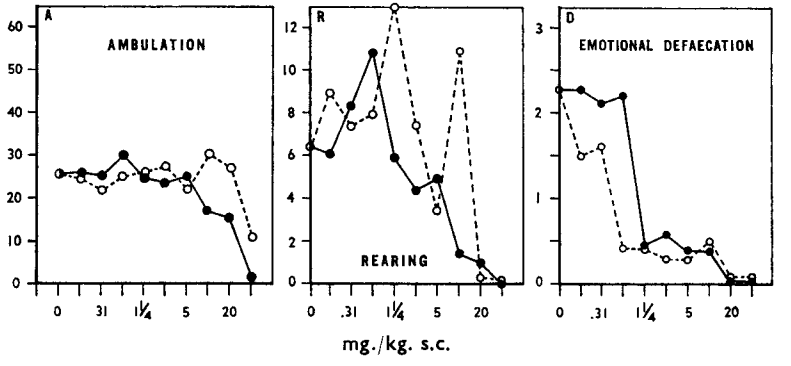


FIG. 3. Influence of R 3365 (●—●) and of morphine (o---o) on ambulation, rearing and defaecation of naive Wistar rats in an unfamiliar open field situation.
Each point represents the average for a group of 8 rats, observed for a period of exactly 3 min. about 1 hr. after dosage.
A = mean ambulation score per rat. R = mean rearing score per rat.
D = mean defaecation score per rat.

Water is available *ad libitum* all the time. Each animal is weighed before and after each eating period to determine weight gain. Food consumption (in g./2 hr.) and faecal excretion (numbers of pellets per 22 hr. after each eating period) are determined as well. After a few weeks of training on this food deprivation schedule the daily values of all three parameters reach surprisingly constant maximal levels, while weight, determined before food consumption, starts to increase gradually and slowly. At this point groups of 5 rats are formed for studying drug effects. After two control days the drug is administered to a group of adequately trained rats by the subcutaneous route, 1 hr. before the usual feeding period of 2 hr. The values are then expressed as percentages of the average values for the two preceding control days (Fig. 4). ED50 values are calculated by probit analysis.

Results. Pirinitramide is about 1·5 times more active than morphine as an inhibitor of food consumption in rats. Its dose-response curve is steeper than the morphine curve. Morphine, on the other hand,

seems to be the more constipating. The following ED50 values (mg./kg.) were calculated by probit analysis.

	Pirinitramide	Morphine
Weight gain	8·7 (6·0–13)	11 (7·1–15)
Food consumed	8·7 (6·3–12)	13 (8·7–20)
Faeces	11 (7·3–15)	11 (6·2–19)

Jumping Box Test in Dogs and in Rats

Method. The avoidance-escape behaviour method for dogs has been described previously by Niemegeers and Janssen (1960). Except for the reduced size of the apparatus, the same method was used for rats.

Results. Pirinitramide, morphine and all other morphine-like drugs we have tested are active only at very high doses in this particular test. Unlike neuroleptic agents they increase the reaction time of avoidance

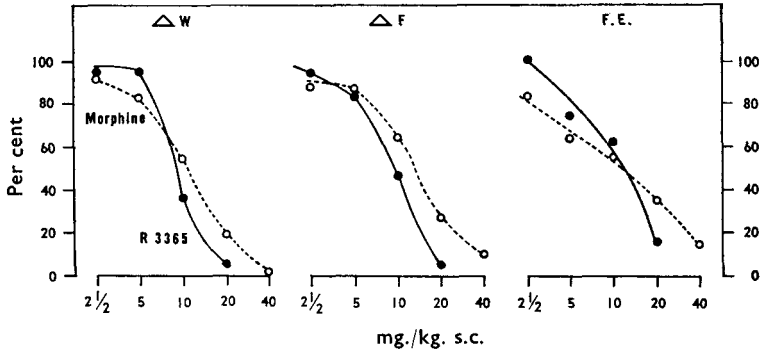


FIG. 4. Influence of R 3365 (●—●) and of morphine (o---o) on weight gain (ΔW), food consumption (ΔF) and faecal excretion (F.E.) of rats trained on a 22 hr. deprivation schedule.

responses only after injection of doses producing overt ataxia and other signs of neurological involvement.

In rats 2·5 mg./kg. s.c. of pirinitramide was inactive. The effects of 5 mg./kg. were very slight. After 10 mg./kg. s.c. some rats failed to avoid shock 1 hr. after injection, but were nearly normal again after 4 hr. Similar effects were obtained with the same doses of morphine.

In dogs 0·31 mg./kg. s.c. of pirinitramide was inactive. A subcutaneous dose of 1·25 mg./kg. had a very slight effect and 5 mg./kg. induced significantly prolonged reaction times in about half of the animals. At this high dose level however an abnormally high frequency of "paradoxical" errors were observed. Maximal effects were again observed 1 hr. after injection. Similar effects were obtained with higher doses of morphine.

Rotating Rod Test

Method. The details of the method have been described previously (Janssen, van de Westeringh, Jageneau, Demoen, Hermans, Van Daele, Schellekens, Van der Eycken and Niemegeers, 1960). Mice are selected

that can maintain equilibrium on a rotating rod (5 revolutions/min.) for at least 3 min. These animals are then injected subcutaneously with a drug under investigation and put again on the rotating rod at various time intervals after dosage.

Results. Like morphine, pirinitramide inhibited the co-ordinated activity required for maintaining equilibrium on a "rotarod" at very high doses only. The ED50 values (mg./kg. s.c., obtained by probit analysis, are pirinitramide, 24(17 — 35) (S = 1·8; fS = 1·4); morphine, 22(15 — 33) (S = 2·2; fS = 1·6). Both drugs were equally active in this test.

Righting Reflex Test in Mice and in Rats

Method. The details of these tests are described by Janssen, van de Westeringh, Jageneau, Demoen, Hermans, Van Daele, Schellekens, Van der Eycken and Niemegeers (1960).

When put on their backs on an undulated metal surface (30°) normal mice and rats show a typical righting reflex immediately after being released. Drugs are given subcutaneously and the animals are put on their backs at fixed time intervals after injection, and the time taken to resume upright posture noted.

Results. Probit analysis gave the following ED50 values (mg./kg.):

	Mice	Rats
Pirinitramide	28 (20–39)	8·9 (6·1–13)
Morphine	45 (30–68)	13 (8·5–19)

These doses are much higher than are required for inducing complete loss of reactivity to noxious stimuli in both species. This is typical for other morphine-like drugs.

Fighting Test in Mice

Method. Aggressive male mice are selected on the basis that they will savagely and immediately attack another male mouse (intruder), after a few days of isolation. (Janssen, Jageneau and Niemegeers, 1960).

Results. Probit analysis shows that pirinitramide and morphine are equiactive at doses lower than the ED50 values (mg./kg.) of the hot plate test: pirinitramide, ED50 = 3·9 (2·0 — 7·6); S = 2·9; fS = 2·2; morphines, ED50 = 4·9 (3·0 — 7·9); S = 5·9; fS = 2·2. Both drugs therefore are relatively selective anti-aggressive agents in mice.

Palpebral Test in Rats

Method. Eight pairs of adult male Wistar rats are selected. One rat of each pair chosen randomly is treated with a subcutaneous dose of drug, the other with solvent. The animals are then put in individual cages provided with a glass window for observation. One hr. after dosage two trained observers independently observe the eyes of each pair of rats without touching the animals. The observers select the rat of each pair having the greatest tendency to close the eyes, *i.e.* the animals having the smaller palpebral aperture. The same score system as described for the

cold and hot plate tests in rats, is then used for expressing the degree of confidence of the observer in his own judgment.

Results. Pirinitramide, morphine as well as many other morphine-like analgesics and CNS-stimulants such as amphetamine, produce exophthalmia in rats. Significant effects were observed in this test with 5 mg./kg. and above of pirinitramide and with 10 mg./kg. and above of morphine. Smaller doses were inactive. Relatively high doses of both drugs are therefore required to produce exophthalmia in the rat and pirinitramide is about twice as active as morphine.

Cornea and Pinna Reflex Tests

Method. Ten adult Wistar rats are subcutaneously treated with a drug and 0·25, 0·5, 1, 2, 4, and 8 hr. thereafter the corneal and pinna reflexes are elicited in the normal way. A rat failing to show a typical reflex at any time after dosage is considered to have temporarily lost that reflex. This never happens in control animals.

Results. Probit analysis shows that the ED50 doses (mg./kg. s.c.) of pirinitramide and of morphine needed to produce temporary loss of the corneal or pinna reflexes in rats are: corneal reflex: pirinitramide, 2·5 (2·1 — 3·0); morphine, 4·0 (2·9 — 5·2); pinna reflex: 4·0 (2·9 — 5·2); morphine, 4·0 (2·9 — 5·2).

Apomorphine Test in Dogs

Method. The method has been described previously (Janssen and Niemegeers, 1959; Janssen, Niemegeers and Schellekens, 1960; Niemegeers, 1960).

A subcutaneous dose of the compound under investigation is followed 30 min. later by a challenging dose of apomorphine 0·31 mg./kg. s.c. This induced emesis in all control experiments.

Results. Most morphine-like drugs produce vomiting in dogs but also block the emetic effects of apomorphine in the same species. As an apomorphine-antagonist in dogs, pirinitramide is about 2·5 times more active than morphine hydrochloride (ED50 values of 0·4 and 1·0 mg./kg. s.c. respectively).

Pirinitramide however is practically devoid of emetic properties at doses of 0·31 to 2·5 mg./kg. s.c., whereas morphine produces vomiting in most dogs at these doses.

Apomorphine-antagonism in Rats

Method. (Janssen, Niemegeers and Jageneau, 1960). Male Wistar rats are isolated in individual cages with shavings on the floor and a glass window for observation. One hr. after a subcutaneous dose of the test drug each rat receives an intravenous injection of 1·25 mg./kg. of apomorphine HCl, and 5, 10 and 20 min. thereafter the animals are observed for about 1 min. by an observer, unaware of the treatment given. The presence or absence of the typical "chewing movements" (compulsory gnawing or "Zwangsnagen") is noted.

Results. The ED50 values (mg./kg. s.c.) obtained by probit analysis were pirinitramide, 23 (16 — 31); morphine, 6·0 (2·9 — 12). Both dose-effect curves differed significantly from parallelism. As an apomorphine-antagonist in rats morphine is about four times more active than pirinitramide.

Amphetamine-antagonism in Rats

Method. Male Wistar rats are isolated in individual cages with shavings on the floor and a glass window for observation. Immediately after a subcutaneous dose of the test drug, each rat receives an intravenous dose of 10 mg./kg. of amphetamine, and 55 and 65 min. thereafter the presence or absence of the typical amphetamine-induced "chewing-movements" ("Zwangsnagen") is noted by an unbiased trained observer, unaware of the treatment given. The probability of occurrence of chewing behaviour in control rats in these conditions is over 95 per cent.

Results. Probit analysis shows that the ED50 in mg./kg. s.c. of pirinitramide was 3·3 (2·3 — 4·7) and of morphine 1·7 (0·78 — 3·7). The dose-effect curves do not significantly deviate from parallelism. The difference between the ED50's is not significant.

Tryptamine-antagonism in Rats

Method. Male Wistar rats are isolated in individual observation cages and subcutaneously treated with the test drug. One hr. thereafter each rat receives an intravenous dose of 40 mg./kg. of tryptamine hydrochloride, which immediately produces, among other effects, typical bilateral clonic convulsions of the forepaws in about 96 per cent of all control rats (Tedeschi, Tedeschi and Fellows, 1959).

Results. At high doses both pirinitramide and morphine are antagonists of the clonic convulsions. The following ED50 values (mg./kg. s.c.) were obtained by probit analysis: pirinitramide, 7·5 (5·4 — 11); morphine, 10 (6·3 — 16). The difference between both values is not significant. Both compounds however are devoid of anticonvulsant activity against leptazol or strychnine in mice.

Nalorphine-antagonism

The well-known morphine-antagonist nalorphine is also a potent antagonist of pirinitramide. An easy way to demonstrate this effect is to give mice, rats or dogs a toxic dose of pirinitramide, followed by an intravenous injection of 0·5 to 10 mg./kg. of nalorphine as soon as the peak effects are observed. Loss of righting reflex, corneal reflex and pinna reflex as well as the bradypnoea, observed after 80 mg./kg. of pirinitramide s.c. in rats, is dramatically antagonised within one min. by 1·25 mg./kg. of nalorphine i.v. Similar results are obtained in dogs treated with 2·5 or 5 mg./kg. of pirinitramide s.c., bradypnoea, ataxia, prostration and insensitivity to noxious stimuli being rapidly abolished by nalorphine.

Respiration

High doses of both pirinitramide and morphine regularly produce striking bradypnoea in mice, rats, dogs and squirrel monkeys. We have studied some of the quantitative aspects of this problem in rats.

Method. A battery of 12 triangular stainless steel cages (7·5 × 7·5 × 18 cm.) in which rats may be immobilised are fixed at the top to a metal bar. The front is made of wire gauze and the back can be closed with a plastic shutter. A special "feeling element," connected to a direct writing four-channel-electrocardiograph, may be fixed in constant contact with the lower part of the thorax of each rat through an aperture on the bottom of the cage. The respiratory movements are inscribed directly on paper (5 mm./sec.).

Results. A randomised 8 × 8 latin square design was used for studying the effects of 2·5, 5·0, 10 and 20 mg./kg. s.c. of pirinitramide and of

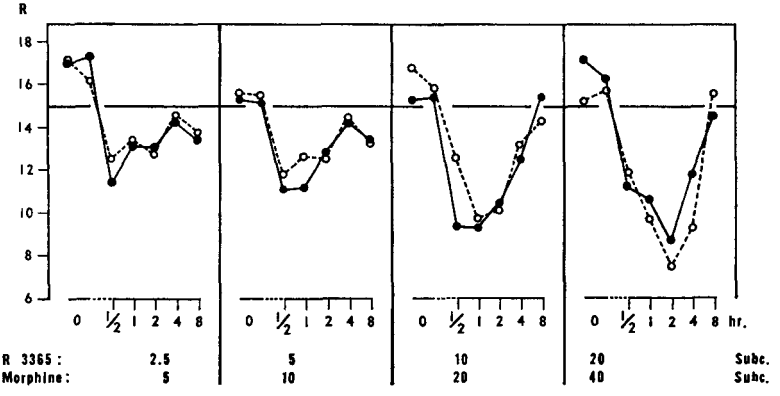


FIG. 5. Influence of R 3365 (●—●) and of morphine (o---o) in mg./kg. on respiratory rate in non-anaesthetised Wistar rats. R = respiratory rate 10^{-3} (frequency/sec./8 rats).

morphine, 5·0, 10, 20 and 40 mg./kg. s.c., in eight adult male Wistar rats. Each animal was treated once a week. After two control readings, the respiratory rate of each rat was recorded over about 30 sec. to 1 min. at ½, 1, 2, 4 and 8 hr. after each dose. There was no evidence of tolerance developing over the experimental period of eight weeks, but much individual variation occurred. As shown in Fig. 5, the time-effect curves of both drugs are similar. Maximal respiratory depression was observed some 30 min. after injection of the smallest doses and 2 hr. after injection of the largest doses. Recovery occurred with comparable speed at all dose levels.

The slopes of both curves are very flat and seem to deviate from parallelism. At the highest dose levels morphine is somewhat more active than pirinitramide as a respiratory depressant in rats, whereas the opposite order of activity is found at low doses. The differences between both drugs are small. Apnoea was not observed in any of these tests. High subtoxic dose levels of both drugs are required to produce a dangerous degree of respiratory depression in the rat.

Mydriatic Activity in Mice

Method. Using a previously described method (Janssen and Jageneau, 1957; Janssen and Jageneau, 1958) pirinitramide was found to be more active than morphine as a mydriatic agent in mice.

Results. Probit analysis gave the following ED50 in mg./kg. s.c.: pirinitramide, 11·6 (8·7–15·5); morphine, 14·9 (14·3–15·5). The ED50 values of most morphine-like analgesics in this test are not significantly different from their respective ED50 values in the hot plate test in mice (Janssen and Jageneau, 1956). For pirinitramide we found a high ratio of about 2.

Blood Pressure and Pressor Effects of Adrenaline and Noradrenaline

Intravenous doses of 0·02 to 0·63 mg./kg. of pirinitramide had no detectable influence on arterial blood pressure in rats and dogs anaesthetised with chloralose. We were unable to prove conclusively that the pressor effects of adrenaline and of noradrenaline (0·0025 mg./kg. i.v. of each) were significantly influenced in the anaesthetised rat by 0·02 to 0·63 mg./kg. of pirinitramide.

There was a suggestion of slight potentiation, particularly of noradrenaline, after administration of the highest doses. This point merits further investigation.

Influence on Adrenaline-induced Mydriasis in Rats

After intravenous injection of 0·04 mg./kg. of adrenaline in normal Wistar rats, a short lasting mydriatic effect is seen in all control rats. The peak effect (about 2 mm.) is obtained within 30 sec. and the total duration of action is about 8 min. Peak effect and duration of this adrenaline induced mydriasis is much enhanced after subcutaneous injection of 10 mg./kg. of both pirinitramide and of morphine.

Acute Toxicity

Method. Groups of 10 adult mice, Wistar rats, cats, dogs and squirrel monkeys were treated with various intravenous, subcutaneous or oral doses of pirinitramide and put in their usual cages. Mortality was recorded 72 hr. after treatment.

Results. Probit analysis gave the following LD50 values (mg./kg.): mice i.v., 34 (23–51); s.c., 280 (230–340); oral, >320; rats i.v., 13 (10–17); s.c., 160 (100–260); oral, 320 (160–640); cats s.c., >10; dogs s.c., >40; monkeys s.c., >10.

After intravenous injection the animals either die within the first hr. or survive, whereas delayed deaths occur as a rule after subcutaneous or oral dosage. Respiratory depression was the main cause of death in all species.

Subacute and Chronic Toxicity in Rats

In one experiment, 12 male and 12 female Wistar rats were selected and divided in 2 × 2 groups of 6 animals. For 15 consecutive days half the animals were given a subcutaneous injection of 1·25 mg./kg. of

pirinitramide daily, the remaining rats being similarly treated with solvent. Weight before treatment and food consumption (standard pellets) was recorded daily. After 15 days all animals were killed and subjected to haematological, pathological and histological examination. In these conditions pirinitramide had no significant effect on growth, food intake: (Fig. 6), haemoglobin content of blood, microhaematocrit values, white blood

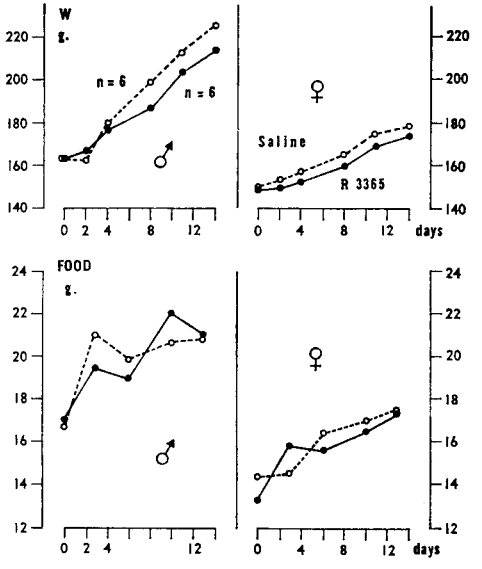


FIG. 6. 15 day subcutaneous rat toxicity of R 3365 (●—●; 1·25 mg./kg. daily) in two groups of 6 male and 6 female young Wistar rats (o---o: 2 × 6 control rats).
W: average daily body-weight per rat in g. FOOD: average daily food consumption (standard pellets) in g./rat.

cells, weight and histology of heart, liver, kidneys, adrenals, pituitary, testes and sex organs. In a second experiment three groups of 10 young male Wistar rats were daily treated with progressively increasing doses of pirinitramide, morphine hydrochloride or solvent for 7 consecutive weeks. Weight was recorded daily immediately before injection. The average weight values (g.) were as follows.

Week	Pirinitramide	Morphine	Solvent
1	1·25 mg./kg.–148 g.	2·5 mg./kg.–148 g.	150 g.
2	2·5 mg./kg.–180 g.	5 mg./kg.–180 g.	185 g.
3	5 mg./kg.–205 g.	10 mg./kg.–200 g.	215 g.
4	10 mg./kg.–226 g.	20 mg./kg.–215 g.	245 g.
5	20 mg./kg.–240 g.	40 mg./kg.–230 g.	265 g.
6	40 mg./kg.–257 g.	80 mg./kg.–232 g.	286 g.
7	80 mg./kg.–270 g.	160 mg./kg.–235 g.	306 g.

It is concluded that both pirinitramide and morphine have a significant growth depressant effect in these conditions, which is probably related to the reduction of food intake caused by these drugs.

Discussion

Pirinitramide, a novel structure lacking some of the classical chemical features of the known morphine-like compounds, has been shown to have typical morphine-like properties in a variety of pharmacological tests. Both pirinitramide and morphine inhibit the reactivity of laboratory animals to various noxious stimuli at doses that are devoid of activity in most other tests. Both drugs also produce the same typical mixture of CNS-depressant and CNS-excitatory effects: excitement is the prominent feature in mice and in cats, while motor activity is mainly depressed in rats, dogs and monkeys. Both substances produce exophthalmia in rats, mydriasis in mice and respiratory depression, loss of corneal-, pinna- and righting-reflexes in all species; at high doses both drugs antagonise some effects of amphetamine, apomorphine and tryptamine, but not of leptazol or strychnine. They are both effectively antagonised by nalorphine.

In most tests in mice, rats and dogs pirinitramide is about twice as active as morphine by the subcutaneous route. In rats it is many times more active orally. It has a quicker onset of action than morphine, but the duration of action of both compounds is about the same. As a respiratory depressant pirinitramide is not more and possibly less active than morphine. Another significant difference seems to be its low emetic activity in the dog.

The available data indicate that the capacity of pirinitramide to produce physical dependence by chronic administration in dogs or its capacity to suppress morphine abstinence signs in the same species, to be extremely low or possibly nil (J. La Barre, private communication and unpublished results from this laboratory).

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