RESEARCH PAPERS

PHARMACOLOGY OF (±)-, (+)- AND (−)-2:2-DIPHENYL-3-METHYL-4-MORPHOLINO-BUTYRYLPYRROLIDINE

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(±)-2:2-Diphenyl-3-methyl-4-morpholino-butyrylpyrrolidine (R610) is a more active analgesic agent in mice, rats and guinea pigs than morphine. The analgesic activity is due to the (+)-isomer (R875). The (−)-isomer (R898) is nearly inactive. R610 lowers the body temperature to below control levels in immobilised rats, inhibits the respiration in unnarcotised rats, lowers the blood pressure in unnarcotised rats with experimental renal hypertension, and causes mydriasis in mice. It also augments the action of barbiturates in mice. In all these respects the (+)-isomer is the active compound of the racemic mixture. The (−)-isomer is almost as toxic as the (+)-isomer, which has a broader therapeutic margin than the (±)-form. The dosage-toxicity relation of highly active analgesic agents in mice and rats is irregular especially in the lower dosage range.

IN previous papers[1,2] the analgesic activities of a series of butyramide derivatives were described. In this paper the pharmacology of the most active of these compounds (±)-2:2-diphenyl-3-methyl-4-morpholino-butyrylpyrrolidine (R610) and of its (+) and (−) forms (R875 and R898) is dealt with in more detail.

EXPERIMENTAL METHODS

Toxicity in Mice and Rats

Acute Toxicity

Intravenous toxicity was estimated in mice by injection of the drugs in saline 0·1 ml./10 g. given over 20 ±2 seconds into the tail veins, the animals being previously subjected to a temperature of 36 ±1° for 10 minutes to dilate the tail veins. Subcutaneous toxicity in mice was determined by injecting the drugs in saline 0·1 ml./10 g. into the neck. For oral toxicity estimations, the drugs were given in a mucilage in 0·1 ml./10 g. weight by stomach tube to mice, the animals being fasted for 14 hours previously. After administration of the drugs the animals were kept at a temperature of 24 ±2° in groups of 10, each group being in a container of 3·5 litre capacity. The observation period was 3 days.

Acute intraperitoneal and subcutaneous toxicities were estimated by injection of the drugs in saline 0·1 ml./100 g. weight into white rats from an inbred strain, weighing from 120–180 g. using 40 to 120 rats for each compound. The animals were kept in groups of 5 at a temperature of 24 ±2° and observed over 3 days. For intravenous toxicity tests the injections were given in saline 0·1 ml./100 g. into the tail vein after the tail had been kept for 30 seconds in water at a temperature to 42–44° to dilate the veins.

Chronic Toxicity

20 female rats weighing 70 ±15 g. received a daily intraperitoneal injection of 1·25 mg. of R875 per kg. weight during a period of five months. Two other groups of 20 were given saline or 10 mg. of morphine per kg. body weight respectively. Growth curves were drawn from weekly weights. At the end of the experiment the rats were killed and the organs examined histopathologically.

Analgesic Activity

Observations on rats

We used a modification of the method of d'Amour and Smith[3] described in a previous paper from our laboratory[4]. A criterion of analgesic activity was derived as follows. In 234 rats the reaction time to a radiant heat stimulus was estimated immediately before and one hour after the intraperitoneal injection of saline 0·1 ml./100 g. body weight. The frequency distribution of the difference between both values was calculated. The mean value amounted to 0·25 seconds and a value of 3 or more was observed only five times in this series. According to the Camp-Meidell inequality[5] an augmentation of the resting threshold value after the injection of the drug of at least three seconds, could be considered as an analgesic effect at the 95 per cent level of significance. The drugs were injected in saline, 0·1 ml./100 g. weight. The frequency of this all-or-none response in series of 10 animals given geometrically progressing doses of the compounds was the basis for the estimation of ED50 values previously described.

Observations on guinea pigs

We used the thermal radiation method of Winder[6] modified by us[7]. The reaction time to a radiant heat stimulus was estimated immediately before and one hour after the intraperitoneal injection of the compounds in saline 0·1 ml./100 g. body weight. In 246 observations in which only saline was injected, the difference surpassed the value of one watt only in 4 instances. According to the Camp-Meidell inequality[5] a difference of at least one watt could be considered as an analgesic effect, at the 95 per cent level of significance.

Tests on mice

These were made with the methods of Haffner[8] and Eddy[9].

Haffner's method

The modification described by Bianchi and Franceschini[10] was used, replacing the artery clip by small clamps adapted from alligator clips.* These have the teeth of the clip replaced by a flat copper surface. Clips were applied to the root of the tail for thirty seconds or until a reaction occurred within this period. Only actual biting the clip was considered as a reaction. Before the experiments the non-reactors were eliminated. In our experience mice which once reacted in the described manner to the

* Mueller Electric Co., Cleveland, 60 series.

application of the clip usually did so again. Only 5 out of 200 mice failed to do so. Disappearance of the biting reaction after the administration of the drug was used as an all-or-none analgesic effect. The frequency of this response in series of ten aminals given geometrically progressing doses of the compounds was again used for the estimation of ED50 values. The drugs were given intravenously, subcutaneously and orally; the interval between the administration of the drug and the application of the clamp was 30 minutes in the first two, and one hour after oral doses.

Eddy's hot plate method[9]

This was slightly modified. The height of the glass cylinder was reduced to 4·5 cm. and covered with a plastic plate, which could be switched on and off by a simple movement. This prevented the mice from walking on their hind legs or from jumping out of the container. The reaction time was the time elapsing between contact with the plate and the licking response. It was determined twice before, with an interval of five minutes, and 10, 20, 30, 40, 50, and 60 minutes after drug administration. The frequency distribution of the sum of the differences between the reaction times after the drug and the average control value was investigated in 444 mice. As the distribution of this value was not normal[11], a criterion for analgesia was derived by using the Tchebycheff inequality[5] (mean value = 0·27 seconds; S.D. = 15·5; a variation of at least 50 seconds being significant at the 19/20 significance level). With this criterion defining a quantal response we used the graphical method of Litchfield and Wilcoxon[12] for the estimation of ED50 values.

Influence on body temperature of immobilised rats

White rats weighing 150 to 200 g. were placed in a container made from small-mesh brass netting divided into ten longitudinal compartments for individual rats. The animals were provided with rectal thermocouples, and temperatures recorded once before and 10 times after the subcutaneous injection of the drugs (in saline 0·1 ml./100 g. body weight) with intervals of 15 minutes. The frequency distribution of the average temperature variation was studied in a series of 221 observations. This average was negative (0·38°), because of the fall of body temperature under the circumstances. The average fall in the ten measurements exceeded a value of 1·5° only 8 times. If this value was exceeded a hypothermic effect was considered to have occurred. The frequency of such an effect in series of ten observations was used for the estimation of ED50 values according to Litchfield and Wilcoxon[12].

Respiration in unnarcotised rats

The rat was placed in a cage of mesh wire with a springsteel tape encircling the thorax. One end of the tape was attached to the top of the cage and the other to the voice coil of a modified loudspeaker system,* functioning as a transducer. The cone was removed from

* Philips AD3500M.

the voice coil to prevent it from picking up noise. By this apparatus the thorax movements were converted into electrical potentials which were led to a penwriter, via a symmetrical R.C. filter circuit with a time constant of 0·1 second.

Four rats weighing from 250–300 g. were put in the apparatus and alternately connected with the recording unit in a fixed sequence. The respiratory movements were recorded for 30 seconds in each animal. If struggling occurred the recording was continued until an uninterrupted respiration curve of 30 seconds duration was obtained. Then the transducer of the next animal was connected with the recording unit. Each five minutes the complete cycle of 4 animals was repeated, until a total of 7 cycles was obtained. Then the intraperitoneal injections were given in saline per 0·1 ml./100 g. body weight. Immediately afterwards the recording was restored until a run of 20 cycles after the injection was completed. In 42 cases in which only the saline was given, the frequency distribution of the difference between the average respiratory frequencies in both periods, expressed as percentage of the average pre-injection frequency, was studied ($\bar{x} = -1{\cdot}2$, S.D. = 3·9). Applying the Camp-Meidell Inequality[5] to these data, an all-or-none criterion for respiratory depression could be derived. We found that a diminution of the average frequency to below 85 per cent of the pre-injection value was statistically significant at the 95 per cent level. The frequency of this effect in series of ten observations under different dosages was used for the estimation of ED50 values as described above.

Influence on the blood pressure in unnarcotised rats with experimental renal hypertension.

The blood pressure was taken with a modification of the tail plethysmograph described elsewhere[13]. Hypertension was induced by constriction of one renal artery and ablation of the other kidney[14]. Influence with drugs were investigated as previously described[15].

Antidiuretic activity in rats

Female rats weighing about 150–250 g. were kept in metabolic cages constructed to collect urine without faeces. Urine not voided spontaneously was obtained by manual suprapubic pressure. This method is practically quantitative, as was demonstrated previously[17]. The rats were fasted for 24 hours but allowed water *ad libitum*. Two hours before the injection of the drug (t_1) the bladder was emptied as described and water 2·5 ml./100 g. body weight was given by stomach tube. Two hours later (t_2) the bladder was again emptied. The total of the volume of urine thus obtained and voided spontaneously during the two hours period was noted. A quantity of 5 ml./100 g. body weight of tepid water was then given by stomach tube and the drug was injected subcutaneously in saline 0·2 ml./100 g. body weight. Urine was collected during one hour and expressed at the end of the experiment (t_3).

The total excretion ($t_1 + t_2 + t_3$) was expressed as the percentage of the water load. This amounted to 7·7 per cent of the body weight minus the quantity of the urine produced between t_1 and t_2.

We studied the frequency distribution of this statistic in 78 observations with saline, 18 with morphine and 15 with R875. A diuresis of less than 10 per cent of the load never occurred in the controls. It was easily obtained with suitable dosages of morphine and R875. We considered urine production of less than ten per cent of the water load to be an antidiuretic effect. ED50 values could thus be obtained as described above.

Mydriatic response in mice

We used the method described by Pulewka[16]. The pupil diameter in white mice of an inbred strain, weighing 16–19 g., was measured by means of a microscope with an eye-piece micrometer. Diameters were measured immediately before and 10 minutes after the intravenous injection of the compounds, in saline 0·1 ml./10 g. body weight. The frequency distribution of the difference between both measurements in 100 mice injected with the saline only, was studied. Application of the Camp-Meidell Inequality[5] enabled us to define an all-or-none mydriatic response, significant at the 95 per cent probability level. We estimated ED50 values in the usual manner.

Augmentation of the effect of barbiturates in mice

White mice weighing 18–23 g. were injected subcutaneously with the analgesic. Fifteen minutes later 10 mg. pentobarbitone sodium per kg. body weight was given intravenously in 0·1 ml. saline per 10 g. body weight. The injection time was standardised at 20 ± 1 seconds. The animals were then put on their back on a warmed surface (30 $\pm 1°$). Mice turning on their feet within 30 seconds were again put on their back. This was eventually repeated twice. If a loss of righting reflexes lasting at least 30 seconds occurred, this was considered as an augmentation of the barbiturate effect. In a series of 80 control observations with saline this all-or-none response never occurred. We computed ED50 values as described above.

TABLE I

ACUTE INTRAVENOUS TOXICITY IN MICE AND RATS
LD50 VALUES IN MG./KG.

Compound	Mice	Rats
R610	25·0 ±4·3 (80)*	12·2 ±2·0 (55)
R875	21·0 ±2·1 (85)	13·0 ±4·1 (80)
R898	30·5 ±5·5 (60)	37·0 ±4·8 (55)
Morphine	151·0 ±20·0 (130)	120 (45)

* Number of animals.

RESULTS

Acute Toxicity

The results of acute toxicity experiments are reported in Table I and Figure 1. The phenomena observed during the work are qualitatively alike for all 4 compounds investigated. Alertness, excitation alternating

with depression, the Straub phenomenon, salivation, convulsions, diarrhoea, haematuria and cyanosis were regularly observed. Death seemed to be due to asphyxia. A rather striking difference between morphine and the synthetics was the degree of katatonia caused in rats. In the case of morphine some rigidity was regularly seen, but high doses of

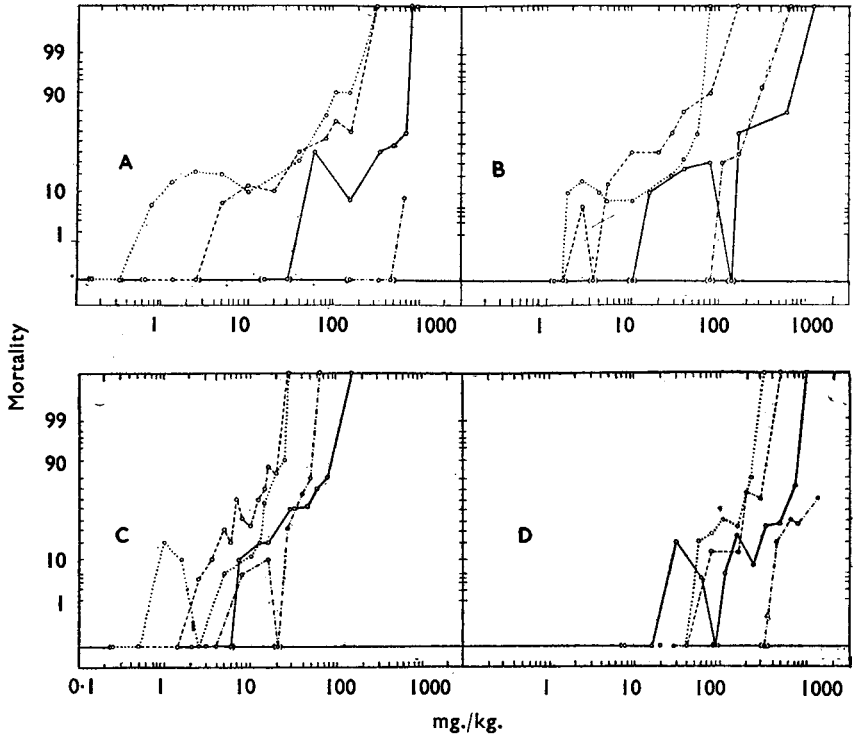

FIG. 1. The acute (A) subcutaneous (B) intraperitoneal toxicity in rats and (C) subcutaneous and (D) oral toxicity in mice. LD50 values. Number of animals in parentheses.

	A	B	C	D
— Morphine	600 (123)	100 (125)	318 ± 130 (140)	450 ± 150 (344)
- - - R610	75 ± 52 (120)	21·3 ± 112 (120)	125 ± 50 (255)	220 ± 75 (135)
... R875	50 ± 30 (178)	(179)	140 ± 60 (160)	168 ± 36 (165)
-.- R898	> 640 (55)	162 ± 13 (97)	330 ± 100 (115)	1000 (90)

R610 and its optical isomers made the animals look as if they were carved from wood.

We also injected a number of unnarcotised dogs and cats with larger than analgesic dosages. Dogs were very much depressed, they showed salivation and bradypnoea, while clonic rapidly reversible convulsions also occurred. Cats became restless and excited, with extreme mydriasis and severe convulsions.

Chronic toxicity

The average growth of the rats is shown in Figure 2. In the control group one rat died, and three in each of the experimental groups. In both experimental groups the animals became progressively aggressive towards the end of the experiments. The growth was slightly inhibited by 10 mg. morphine per kg. body weight and by 1·25 mg. R875 per kg. body weight. No gross or miscroscopic alterations were found in a pathological investigation of liver, kidneys, brain, bone-marrow, spleen and heart muscle.

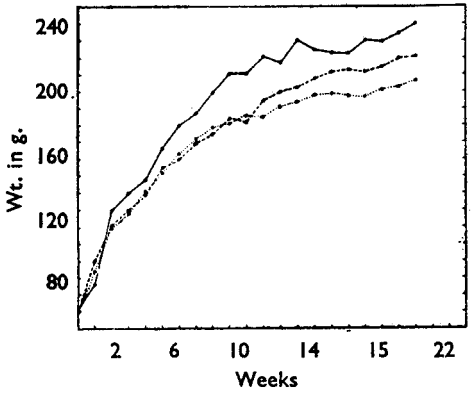

FIG. 2. Growth curves of rats in a subacute toxicity experiment. — saline; - - - R875, 1·25 mg./kg. i.p.; . . . morphine 10 mg./kg. i.p.

Analgesic activity

The results obtained are given in Table II. According to each of the four criteria used R610 is much more active than morphine. The activity of the racemic mixture is to be ascribed to the (+)-isomer, its optical isomer being practically inactive.

TABLE II

ANALGESIC ACTIVITY IN MICE, RATS AND GUINEA PIGS
ED50 VALUES IN MG./KG.

Compound	Hot plate Eddy mice s.c.	Haffner method mice i.v.	d'Amour and Smith rats i.p.	Winder guinea pigs i.p.
R610	1·7 ±0·5 (120)*	0·62 ±0·3 (60)	2·25 ±0·8 (90)	1·4 ±0·5 (80)
R875	0·87 ±0·3 (140)	0·35 ±0·3 (40)	1·3 ±0·4 (120)	0·79 ±0·3 (85)
R898	85 ±19·6 (80)	18 ±5 (40)	116 ±48·7 (54)	81 ±35·6 (35)
Morphine	10·2 ±1·2 (389)	3·79 ±0·7 (200)	7·58 ±0·9 (340)	9·85 ±3·0 (138)

* Number of animals.

Influence on the body temperature, diuresis and respiration in rats, on the pupil diameter in mice and on the pentobarbital effect in mice

The results are given in Table III. The potency ratios are the same as in the analgesia experiments. The pharmacological activity of R610 is to be ascribed to its (+)-isomer.

The activity of the (−)-isomer was greater in the very sensitive mydriatic test than by the other criteria. A dose of 0·11 mg. per kg. body weight R875 (ED50) was given to 60 mice, 8·0 mg. R898 per kg. body weight (ED50) to a second group, and 0·055 mg. R875 per kg. body weight (½ ED50) together with 4·0 mg. R898 per kg. body weight (½ ED50) to a third group also of 60 mice. The numbers of mydriatic responses in the three groups were 26, 31 and 39 respectively. According to the

TABLE III

ED50 VALUES (IN MG./KG. OF THE BASE) OF MORPHINE AND R875 AND R898

Compounds	Hypothermic activity in rats	Depression of respiration in rats	Antidiuretic activity in rats	Augmentation of pentobarbitone effect in mice	Mydriatic activity in mice
R610	1·28 ±0·4 (48)*	1·8 ±0·8 (58)	—	1·25 ±0·4 (76)	0·24 ±0·1 (59)
R875	0·87 ±0·2 (62)	1·3 ±0·4 (77)	0·37 ±0·06 (59)	1·1 ±0·2 (182)	0·11 ±0·1 (120)
R898	inactive up to 40 mg./kg. (30)	inactive up to 40 mg./kg. (20)	—	inactive up to 16 mg./kg. (40)	8·0 ±1·5 (69)
Morphine	13·6 ±3·7 (50)	7·2 ±2·9 (50)	2·7 ±0·4 (88)	21·2 ±5·3 (79)	0·56 ±0·15 (130)

* Number of animals.

χ square test this result does not contradict the supposition of simple addition ($P \approx 0{\cdot}15$).

Effect on the blood pressure of unnarcotised rats with experimental renal hypertension

The results are given in Figure 3. R898 was again nearly inactive. All compounds lowered the blood pressure.

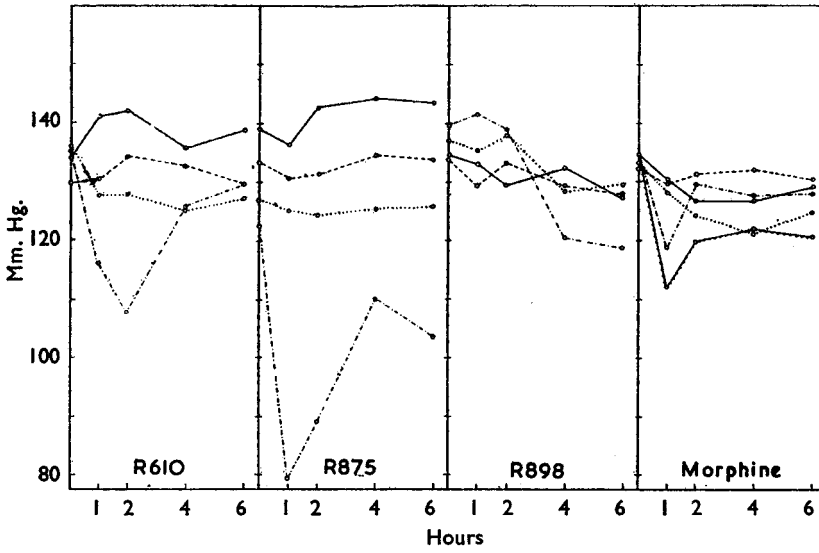

FIG. 3. Influence on blood pressure of rats with experimental renal hypertension. Number of animals in parentheses.

R610, - - - saline (16); — 0·625 mg./kg. (7); . . . 1·25 mg./kg. (8); - - - 2·5 mg./kg. (8)
R875, „ „ (31); „ „ „ (7); „ „ „ (15); „ „ „ (16)
R898 „ „ (8); „ 10 mg./kg. (8); „ 20 mg./kg. (8); „ 40 mg./kg. (8)
Morphine „ (39); „ 5 „ „ (8); „ 10 „ (20); „ 20 „ (20); 40 mg./kg. (13).

Effect on the blood pressure and respiration in allobarbitone narcotised cats and urethane narcotised rats; Nalorphine antagonism

Morphine and R875 both depressed the respiration and caused a fall of the blood pressure. Nalorphine in suitable dosages antagonised these effects.

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REFERENCES

1. van Proosdij-Hartzema and de Jongh, *Acta Physiol. Pharmacol. Neerl.*, 1957, in the press.
2. Janssen, *J. Amer. chem. Soc.*, 1956, **78**, 3862.
3. D'Amour and Smith, *J. Pharmacol.*, 1941, **72**, 74.
4. De Jongh and Knoppers, *Arch. int. Pharmacodyn.*, 1952, **90**, 466.
5. Davies, *Statistical Methods in Research and Production*, Oliver & Boyd, 1947, 56.
6. Winder, Pfeiffer and Maison, *Arch. int. Pharmacodyn.*, 1946, **72**, 329.
7. De Jongh and Knoppers, *ibid.*, 1952, **90**, 449.
8. Haffner, *Dtsch. med. Wschr.*, 1929, **55**, 731.
9. Eddy and Leimbach, *J. Pharmacol.*, 1953, **107**, 385.
10. Bianchi and Franceschini, *Brit. J. Pharmacol.*, 1954, **9**, 280.
11. Geary and Pearson, *Tests of Normality. Biometrika Off. Univ. Coll. Lond.*, Cambridge University Press, 1938.
12. Litchfield and Wilcoxon, *J. Pharmacol.*, 1949, **96**, 99.
13. Van Proosdij-Hartzema, *Acta Physiol. Pharmacol. Neerl.*, 1954, **3**, 472.
14. Van Proosdij-Hartzema and de Jongh, *ibid.*, 1955, **4**, 37.
15. De Jongh and van Proosdij-Hartzema, *ibid.*, 1955, **4**, 153.
16. Pulewka, *Arch. exp. Path. Pharmak.*, 1932, **168**, 307.
17. De Jongh and Kok, *Acta Physiol. Pharmacol. Neerl.*, 1955, **4**, 222.