RESEARCH PAPERS

A NEW SERIES OF POTENT ANALGESICS:

Dextro 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine and related basic amides

PART II

COMPARATIVE ANALGESIC ACTIVITY, ACUTE TOXICITY AND TOLERANCE DEVELOPMENT IN RATS FOR R875, MORPHINE, PETHIDINE AND METHADONE

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The analgesic activity, acute toxicity and development of tolerance of R875, morphine, pethidine and methadone after subcutaneous administration in rats have been measured. In this animal, R875 is shown to be several times more active than the three other compounds. R875 has a faster onset of action, higher therapeutic ratios and a slower rate of development of tolerance.

SOME pharmacological properties of a new series of secondary and tertiary basic amides[1] were described in part I of this series[2]. The dextrorotatory isomer of 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine, serial number R875, was shown to be the most active analgesic tested, and therefore was selected for further study.

This paper describes some comparative pharmacological and toxicological investigations in rats with R875, morphine, methadone and pethidine.

The following substances were administered subcutaneously to rats of an inbred Wistar strain:

R875: the dextrorotatory isomer of 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine was used as its (+)-tartrate; all doses are expressed in terms of the base; morphine hydrochloride; pethidine hydrochloride; (±) methadone hydrochloride; nalorphine base.

METHOD

Acute Toxicity

Male rats, weighing from 200 to 400 g. were used. At 10 a.m., groups of 10 animals were injected with 1 ml./kg. of an aqueous solution, containing R875, morphine, methadone or pethidine. They were kept in individual containers at room temperature 18°–22°, and observed at 1, 6, 12, 24 hours, 2 and 3 days after injection. Food and water was freely available. A minimum of 5 groups of 10 rats per dose, and 6 dose levels per compound were used.

Analgesic Activity

The analgesic activity was determined using the "hot plate" method, described in part I[2].

Tolerance

Groups of 10 male rats, weighing from 125 to 175 g. were used. Every day at 10 a.m. (except Sunday) the animals were injected with 1 ml./kg. of an aqueous solution containing R875, morphine or pethidine. Five and 10 minutes before, and 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after each dose, the reaction time of the animals was measured using the "hot plate" method[2].

The rats were kept on a liberal diet in individual containers at room temperature 18°–22°. The definition of "positive response" is given in part I of this series.

RESULTS

Acute Toxicity

The results obtained by using 660, 400, 300 and 310 animals and 10, 8, 6 and 7 dose levels respectively are summarized in Table I and Figure 1.

TABLE I

ACUTE TOXICITY IN RATS

(Subcutaneous injection; observation period: 3 days)

	R875			Methadone			Morphine			Pethidine		
	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†
	0·78	0	60	6·25	0	50	12·5	0	50	50	0	50
	1·56	6·7	60	12·5	2	50	25	2	50	100	2	50
	3·13	15·6	90	17·7	12	50	50	16	50	200	10	50
	6·25	25·6	90	25	30	50	100	4	50	250	12	50
	12·5	24·6	110	50	46	50	200	6	50	300	50	50
	25	20	90	100	96	50	400	34	50	400	80	50
	50	40	70			—	800	62	50	800	100	10
	100	65	40			300	1600	84	50			—
	150	66·7	30						—			310
	200	90	20						400			
			660									
LD10		2			16·5			37			210	
LD50		74			54			590			315	
LD50: LD10		37			3·3			16			1·5	
ED50		0·38			5·14			14·3			41·0	
ED99		0·802			14·5			38·0			108	
LD50: ED50		195			10·5			41			7·7	
LD10: ED50		5·3			3·2			2·6			5·1	
LD10: ED99		2·4			1·1			1·0			1·9	

* Per cent mortality. † Number of rats injected.

Inspection of the figure shows that each "log dose-probit mortality" curve has its own particular shape. For R875 there is no detectable relation between dose and mortality within the 3 to 30 mg./kg. dose range, all four doses giving 15 to 25 per cent mortality. Mortality figures of 4 to 16 per cent are obtained with 30 to 300 mg./kg. morphine, 50 mg./kg. morphine being more toxic (16 per cent) than 100 to 200 mg./kg. (4 to 6 per cent). The pethidine- and methadone-curves are characterized by an inflexion around 40 and 7 per cent mortality respectively. Above 50 per cent mortality all curves are fairly linear, but not parallel. The acute subcutaneous toxicity of these compounds therefore cannot be fully described by one single mathematical symbol.

We propose to use the ratio LD50:LD10 for this purpose, the LD50 value symbolizing the mortality at high dose levels and LD10 the mortality at low dose levels.

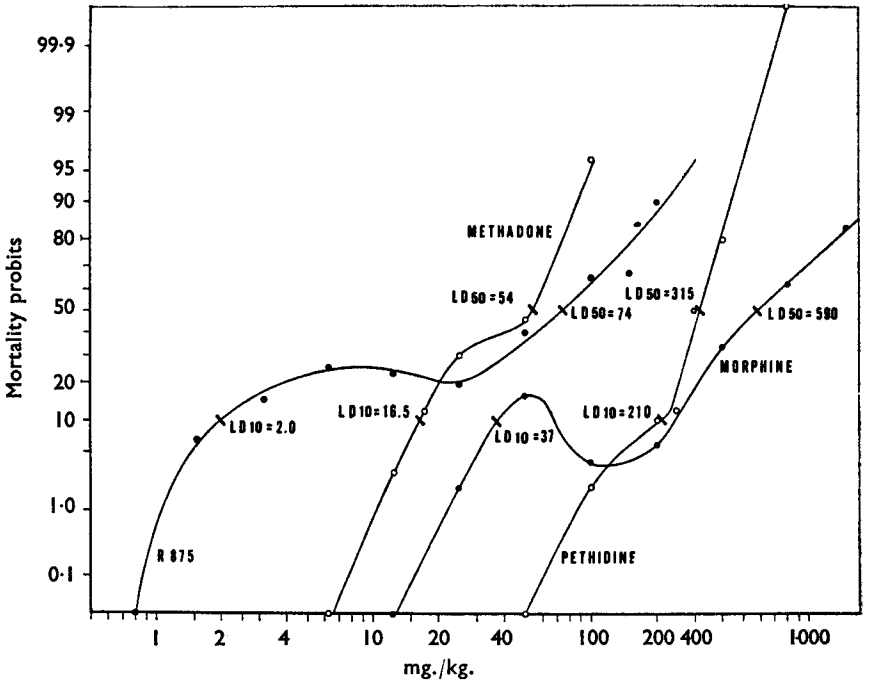

FIG. 1. Acute subcutaneous toxicity in rats (observation period: three days).

For R875 we thus obtain a value of 74:2 = 37, for morphine 590:37 = 16, for methadone 54:16·5 = 3·3 and for pethidine 315:210 = 1·5. A subcutaneous dose of 10 mg./kg. nalorphine produces notable protection against death occuring after simultaneously injected low doses of R875, morphine and methadone (Table II), but not against high doses. In all instances the LD50:LD10 ratios are sharply reduced. Death occuring after low doses thus seems to be caused by a mechanism, which is antagonised by nalorphine, for example respiratory depression.

TABLE II

ACUTE SUBCUTANEOUS TOXICITY IN RATS OF R875, MORPHINE, PETHIDINE AND METHADONE WITH AND WITHOUT NALORPHINE

Substance	mg./kg.	Without nalorphine		With 10 mg./kg. nalorphine	
		Mortality per cent	*n**	Mortality per cent	*n**
R875	6·25	25·6	90	0	50
	50	40	70	4	50
	200	90	20	100	50
Morphine ..	50	16	50	0	50
	550	50	50	2	50
	1600	84	50	82	50
Pethidine ..	300	50	50	60	50
Methadone ..	50	46	50	10	50
	100	96	50	88	50
Nalorphine ..	10	0	50	—	—

* Number of rats injected.

Analgesic Activity

Using the all-or-none criterion of effect, described in the first part of this series, the "total analgesic activity" of R875, morphine, methadone and pethidine was evaluated using a total of 1037 rats.

According to this criterion, R875 was found to be 94 to 124 times more active than pethidine as an "analgesic" in rats, 34 to 42 times more active than morphine, and 12 to 16 times more active than methadone (Table III).

Onset, peak and duration of action are graphically represented in Figure 2, showing the percentage of rats with a reaction time of 30 or more

TABLE III

ANALGESIC ACTIVITY IN RATS

(Subcutaneous injection)

	R875			Methadone			Morphine			Pethidine		
	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†
	0·25	12	50	3·5	20	40	1	0	10	25	12	50
	0·35	33·3	30	5	47·5	40	2·5	0	10	37·5	41·7	48
	0·50	80	60	7·5	80	30	5	2·5	40	50	66·1	59
	1·0	100	99			—	10	20	50	75	93·2	44
	2·0	100	56			110	15	46·6	58			—
	4·0	100	54				20	89·3	56			201
	5·0	100	9				25	98·8	80			
			—				50	100	54			
			358				100	100	10			
									—			
									368			
ED50	0·38 (0·35–0·42)			5·14 (4·59–5·76)			14·3 (13·0–15·7)			41·0 (36·6–45·9)		
fED50	1·10			1·12			1·10			1·12		
S	1·39 (1·32–1·46)			1·56 (1·30–1·87)			1·54 (1·48–1·60)			1·51 (1·34–1·71)		
fS	1·05			1·20			1·04			1·13		
PR	1·0			0·074 (0·064–0·085)			0·027 (0·024–0·030)			0·0093 (0·0081–0·011)		
PR	13·5 (11·8–15·6)			1·0			0·36 (0·31–0·41)			0·125 (0·11–0·15)		
PR	37·6 (33·6–42·1)			2·78 (2·42–3·20)			1·0			0·35 (0·30–0·40)		
PR	107·9 (93·8–124·1)			7·98 (6·82–9·33)			2·87 (2·49–3·30)			1·0		

* Rats, per cent, showing a "positive analgesic response". † Number of rats surviving the experiment.

seconds ("complete analgesia") at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after subcutaneous injection of R875, methadone, morphine and pethidine.

Maximal effects are observed 20 minutes after R875, about 45 minutes after pethidine and about 60 minutes after methadone and morphine. Using atoxic doses, only R875 and, to a lesser extent, pethidine are active 10 minutes after injection.

The duration of action of these analgesics increases with increasing dosage. Two hours after injection, half of the rats showed reaction times of 30 seconds or more at approximately the following dose-levels: 2 mg./kg. R875, 10 mg./kg. methadone, 20 mg./kg. morphine and 100 mg./kg. pethidine. In view of the different shapes of the time-effect curves (Fig. 2), the duration of action of the substances studied, cannot be adequately expressed using simple mathematical symbols. It would

be desirable to make a comparative study of the rates of increase of ED50 values determined at increasing time intervals after injection[6].

Therapeutic Ratios

Using the ED50, ED99, LD50 and LD10 values, obtained as described above, the relative toxicity of R875, morphine, methadone and

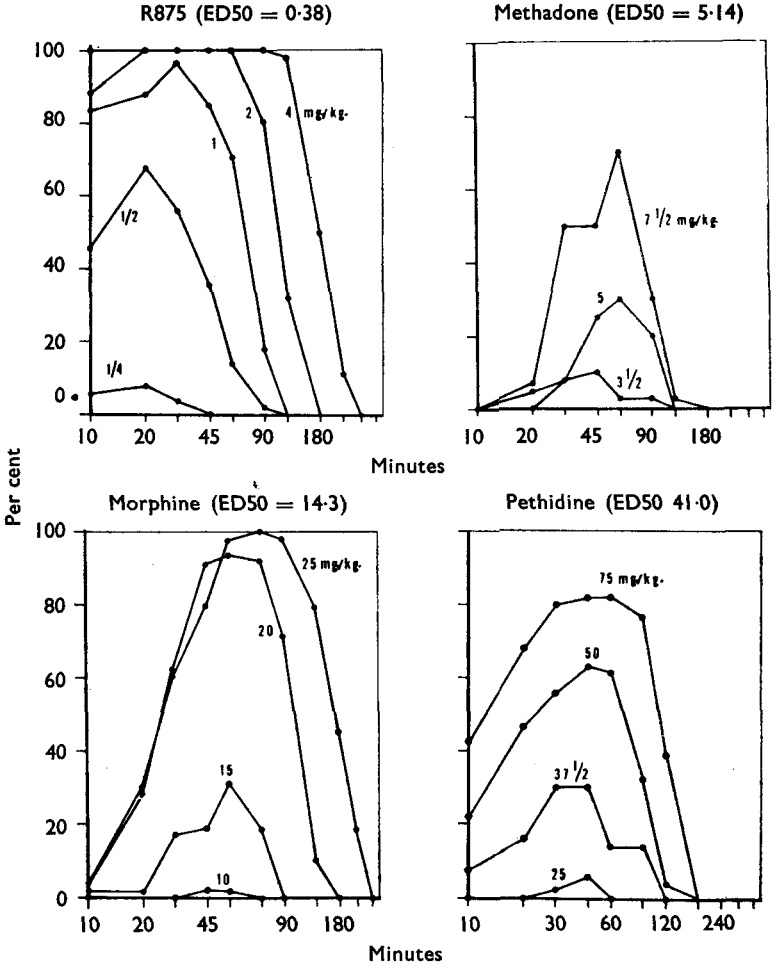

FIG. 2. Duration of "complete" analgesia in rats. (The animals per cent, showing a reaction time greater than 30 seconds at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after subcutaneous injection of R875, morphine, methadone and pethidine.)

pethidine may be expressed by the following "therapeutic" ratios: LD50:ED50, LD10:ED50 and LD10:ED99.

These ratios, tabulated in Table I, show R875 to possess a lower relative toxicity than morphine, methadone and pethidine, regardless of the criterion used.

In order of increasing relative toxicity, these four analgesics may be ranked as follows:

	LD50:ED50	LD10:ED50	LD10:ED99
R875	1	1	1
Morphine ..	2	4	4
Methadone ..	3	3	3
Pethidine ..	4	2	2

Tolerance

The rate of development of tolerance in rats was studied using the following doses, which are approximately equipotent:

R875 .. .. .. 1 mg./kg. (ED99·9 : LD0·4)
Morphine .. .. .. 25 mg./kg. (ED98·8 : LD2·0)
Pethidine .. .. ..100 mg./kg. (ED98·5 : LD2·0)

In the first series of experiments a first group of 20 rats received daily injections of R875 (26 days), a second group of 40 rats were daily injected

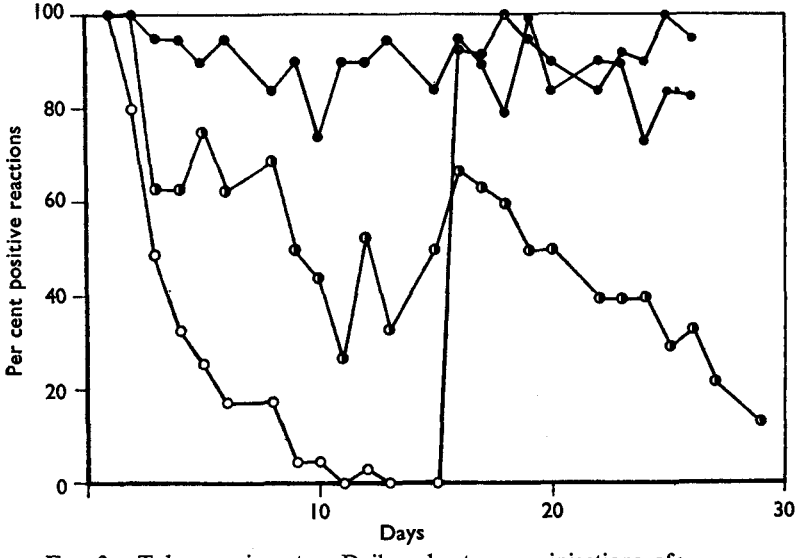

FIG. 3. Tolerance in rats. Daily subcutaneous injections of:
1 mg./kg. R875 (●—●),
25 mg./kg. morphine (○—○), or
100 mg./kg. pethidine (◑—◑).

with morphine (15 days) and a third group with pethidine (20 rats; 29 days).

From the 16th until the 26th day the rats of the morphine-group received daily injections of R875.

As shown in Figure 3, R875 induces much less tolerance than pethidi,ne and pethidine much less than morphine. After 10 days of treatment all animals of the morphine group were completely tolerant, as compared with 50 to 60 per cent tolerance in the pethidine-group and about 10 per cent in the R875 group.

There was little or no cross-tolerance between R875 and morphine, the morphine-tolerant rats responding normally to R875.

With R875, 19 rats out of 20 survived the experiment after 26 days of treatment; after 15 days of treatment with morphine, 38 out of 40 animals survived. Considerable mortality occurred however in the pethidine group, 80 per cent of the rats surviving after 10 days of treatment, 50 per cent after 20 days and only 35 per cent after 29 days. In contrast with R875 and morphine, pethidine caused pronounced local irritation, making it very difficult to inject the animals after a few days of treatment.

The "average weight"-figures of these three groups were computed as follows:

	R875	Morphine	Pethidine
1st day ..	145 g.	165 g.	173 g.
5th day ..	159 g. (+14 g.)	172 g. (+ 7 g.)	170 g. (− 3 g.)
10th day ..	173 g. (+28 g.)	176 g. (+11 g.)	182 g. (+ 9 g.)
15th day ..	184 g. (+39 g.)	184 g. (+19 g.)	195 g. (+22 g.)
20th day ..	190 g. (+45 g.)	—	199 g. (+26 g.)
26th day ..	200 g. (+55 g.)	—	202 g. (+29 g.)

The growth of the animals was obviously much less retarded with R875 than with morphine or pethidine.

The fact that R875-tolerance develops at a much slower rate than morphine-tolerance, is further demonstrated in Figure 4. Sixty rats

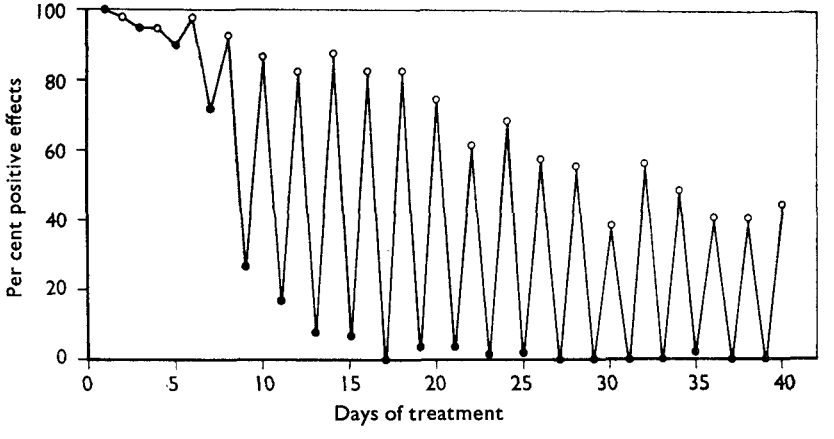

FIG. 4. Tolerance in rats: alternative subcutaneous injections of morphine and R875 in 60 rats.

○—○ R875, 1 mg./kg.
●—● Morphine, 25 mg./kg.

were given subcutaneous doses of morphine (odd days) and of R875 (even days). 49 rats survived after 40 days of treatment. At the end of two weeks all animals were completely tolerant to morphine, whereas 80–90 per cent of the animals still responded to R875.

At the end of 40 days of treatment, about half of the animals were still fully sensitive to R875. Comparing Figures 3 and 4, the impression is gained that tolerance to R875 develops at a faster rate when morphine is

administered on alternate days. It should be noted that R875 does not seem to induce tolerance in man, daily treated for prolonged periods of time[4,5].

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