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Synthetic Anti-diarrhoeal Agents—I. Some Pharmacological Properties of R 1132 and Related Compounds

PAUL A. J. JANSSEN, ANTON H. JAGENEAU and JULES HUYGENS

Research Laboratories Dr. C. Janssen, Beerse (Turnhout), Belgium

Diarrhoea may be caused by neoplasm, protozoa, bacteria, moulds, viruses, drugs, worms, allergies, radiation, vitamin deficiencies and other specific agents.[1] Effective specific therapy is highly desirable but, unfortunately, often unavailable. Most patients with diarrhoea do not suffer from known or recognized organic diseases and in the majority of cases, therefore, symptomatic relief is required. A limited degree of symptomatic relief is provided by psychotherapy, insoluble bismuth powders, clay-like materials, e.g. kaolin, alkaline and buffering agents, substances which form gels, pectin, parasympatholytics[2] and dietary treatment.

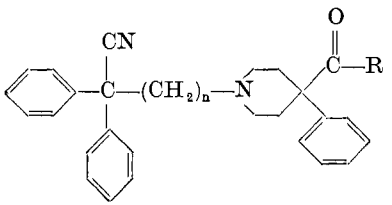
Opium, morphine and its congeners which reduce the rate and intensity of intestinal peristalsis are by far the most effective agents in the symptomatic treatment of diarrhoea. There is however, considerable danger of addiction if they are used continuously[1] and effective doses often cause unpleasant side-effects.

In the course of previous studies[3] we noted that the correlation between 'analgesic' and 'constipating' activity of several hundreds of analgesically active substances is rather poor. Some very active analgesics, e.g. dextromoramide (R 875, Palfium N.D.), produce significant inhibition of the rate of gastro-intestinal propulsion at high doses. Constipation does not seem to be one of the side-effects of this drug in man. Codeine, on the other

hand, is known to produce constipation in man and in animals at relatively small dose levels.

It therefore seemed reasonable to assume that analgesic type compounds devoid of analgesic action but behaving as highly active inhibitors of gastro-intestinal propulsion and defaecation might be synthesized. We therefore screened for 'analgesic' and 'constipating' activity a large number of synthetic compounds which are chemically related to the known synthetic analgesics, using two techniques described in a previous paper,[3] i.e. the 'hot plate' method in mice, and the 'charcoal meal test' in mice.

The purpose of this paper is to describe the results of these and related pharmacological screening methods applied to a new series of compounds I (the R 1132 series) which were synthesized[4] in this laboratory.



I

COR = ester or ketone

n = 2, 3 or 4

In the experimental conditions to be described these compounds are devoid of significant analgesic or parasympatholytic activity when injected subcutaneously in mice and rats. Most of them, however, are more active in reducing the rate of defaecation and gastro-intestinal propulsion in the same species than any other known compounds thus far tested in this laboratory.

Methods

Analgesic and Mydriatic Activity in Mice and Rats

Analgesic activity in mice and rats after subcutaneous administration was evaluated using two modifications of Eddy's hot plate method, which were described in detail in a previous paper.[3]

Mydriatic activity in mice after subcutaneous injection was evaluated using a modification of Pulewka's method.[3,5]

The 'Charcoal Meal' Test in Mice

One hour after an intraperitoneal dose of the substance under investigation, a standardized charcoal suspension was given to groups of ten mice.

The substance is considered to have produced a significant reduction of the rate of gastro-intestinal propulsion when the appendix of the animals contains no charcoal 2 h after the administration of the charcoal meal. The details of this procedure are described in a previous paper.[3]

The Influence of Drugs on the number of Faecal Pellets passed by Wistar Rats

Each experiment involves five groups of 30 female Wistar rats 2–6 months of age and lasts for about one week without interruption. These 150 rats are divided into five groups according to body weight. The following morning the animals are put in special cages ($40 \times 18 \times 18$ cm; bottom and walls of 8 mm mesh; three rats per cage). The faecal pellets are quantitatively collected on a plastic tray, uncontaminated with urine, food or drinking water. The cages are housed in a sound-proof air-conditioned room ($21 \pm 0{\cdot}5°$; 60 ± 5 per cent humidity), provided with constant illumination. Water and food are available *ad libitum* throughout the experiment.

Starting on the third day at 5 p.m., the number of faecal pellets passed by the 3 rats of each of the 50 cages is recorded every sixth hour, 5 p.m., 11 p.m., 5 a.m., and 11 a.m., until the eighth morning at 5. At this point the body weight of each animal is again determined and the next experiment starts.

At 5 p.m. on the fourth day each animal is weighed and subcutaneously, intraperitoneally or orally treated with 10 ml of solvent per kg of body weight.* One group of 30 rats, the control-group, is treated with the solvent only, while the remaining four groups of 30 rats are treated with solvent containing calculated amounts of the substances under investigation. At the end of each experiment, the available information thus includes the

* The members of the R1132 series are poorly soluble in aqueous solution. In these experiments they were administered in aqueous suspension, prepared by mixing and stirring the finely divided powder with water containing 4 drops of Tween 80 for every 100 ml.

number of faecal pellets passed by 50 groups of 3 rats during 18 periods of 6 hours, 4 periods before and 14 periods after administration of the drugs. This means a total of 900 individual figures. These figures are conveniently summarized and symbolized by calculating the total amount of faecal pellets per group of 30 rats and per period of 6 or 24 h (5 p.m.–5 p.m.). The significance of the differences may be conveniently evaluated using conventional rank correlation methods.[6]

The average number of faecal pellets, per group of 30 rats and per 24 h period, passed by 20 such groups treated with solvent only during 4 successive periods of 24 h was 1,154. The 80

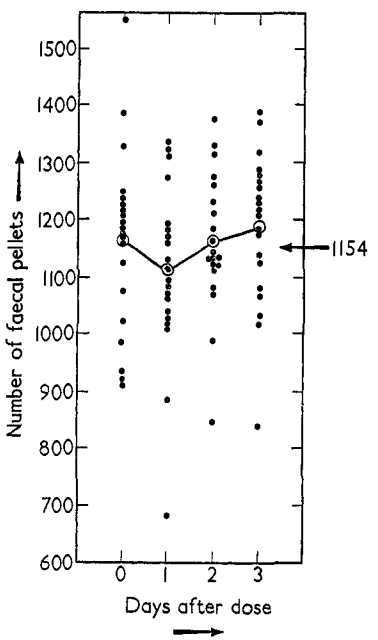


Fig. 1. Faecal pellets passed by Wistar rats. Each black dot represents the total number of faecal pellets passed by one group of 30 rats during a period of 24 h (1 day before and 3 days after administration of various solvents). The open circles represent the averages of 20 consecutive experiments.

day	averages
0	1,163
1	1,106
2	1,160
3	1,189
0–3	1,154

individual results from which this average of 1,154 was derived are graphically represented in Fig. 1, in which each black dot represents the total daily faecal output of one group.

It was found that some groups had a significantly higher faecal output than others throughout the experimental period of 4 days. The correlation between average body weight and average daily faecal output was not significant.

Administration of the solvent resulted in a slightly but significantly decreased faecal output during the day following administion in 2 out of 20 experiments. Only once in these 80 daily periods, however, was the total number of pellets less than 800; 4 times out of 80 it was less than 900 (Fig. 1).

Significant diurnal variation in faecal output was observed in all untreated groups of rats. Analysis of the 20 control experiments described above gave the following results:

6 h period	Average number of pellets per group of 30 rats
5 p.m.–11 p.m.	232
11 p.m.–5 a.m.	338
5 a.m.–11 a.m.	351
11 a.m.–5 p.m.	234

The rate of faecal output in rats is maximal at night. The influence of drugs on the faecal output of rats therefore depends somewhat on the time of administration.

Results

Analgesic and Mydriatic Activity

The 18 compounds of the R1132 series, listed in Table I, were devoid of significant analgesic activity in mice and rats at the subcutaneous dose levels of 10, 20, 40 and 80 mg/kg body weight. At these dose levels the compounds failed to produce a significant degree of mydriasis in mice. All animals were alive at the end of these experiments and showed an apparently normal behaviour.

Table I. Influence of drugs on the rate of gastro-intestinal propulsion of a charcoal meal in mice

	Serial number	Chemical Structure I (R 1132 series)		ED50 with P = 0·05 fiducial limits (in mg/kg I.P.)	Slope and ~ value		Number of mice used
		R	*n*		S	f_s	
1	R 1326	OCH_3	2	2·21 (1·5–3·3)	3·60	1·60	98
2	R 1132	OC_2H_5	2	0·80 (0·55–1·2)	3·35	1·37	148
3	R 1305	OC_2H_5	3	8·9 (4·8–17)	3·80	1·61	75
4	R 1440	OC_2H_5	4	74·0 (55–99)	3·16	1·66	149
5	R 1260	OC_3H_7-*n*	2	0·43 (0·29–0·62)	4·78	1·46	179
6	R 1318	OC_3H_7-*n*	3	12·4 (8·6–18)	8·48	1·64	330
7	R 1261	OC_3H_7-*i*	2	3·52 (1·9–6·6)	5·67	2·40	88
8	R 1416	OC_3H_5[a]	2	0·16 (0·11–0·23)	3·50	1·35	158
9	R 1319	OC_4H_9–*n*	2	0·20 (0·10–0·39)	13·9	1·91	194
10	R 1355	OC_5H_{11}-*n*	2	0·43 (0·25–0·71)	6·29	1·67	189
11	R 1493	OC_6H_{13}-*n*	2	0·46 (0·23–0·92)	13·4	4·10	120
12	R 1357	OC_6H_{11}[b]	2	0·95 (0·55–1·7)	6·39	2·10	118
13	R 1500	OC_7H_{15}-*n*	2	0·60 (0·40–0·89)	6·58	1·59	214
14	R 1375	O-$CH_2CH_2C_6H_5$	2	0·17 (0·12–0·24)	2·64	1·25	137
15	R 1301	C_2H_5	2	17·4 (13–24)	4·54	1·45	268
16	R 1316	C_2H_5	3	11·0 (7·2–17)	3·34	1·39	123
17	R 1302	C_3H_7-*n*	2	23·4 (13–41)	4·14	3·10	68
18	R 1320	C_3H_7-*n*	3	6·4 (4·3–9·6)	4·18	1·45	128
19	morphine hydrochloride			9·0 (6–13)	7·47	1·58	279
20	codeine phosphate			32·5 (20–52)	2·16	1·98	30
21	atropine sulphate			16·5 (12–23)	6·14	1·99	221
22	papaverine hydrochloride			95 (74–123)	2·19	1·33	100

[a] OC_3H_5 = O-allyl. [b] OC_6H_{11} = O-*cyclo*hexyl.

Charcoal Test in Mice

The compounds are all more active than papaverine in the charcoal meal test in mice; 17 of them are more active than codeine, 16 are more active than atropine, and 13 are more active than morphine. In general the esters are more active than the ketones.

The activity of the esters decreases when the side-chain is lengthened from $n = 2$ to $n = 3$ or 4, whereas the ketones become more active. The influence of the structure of the ester group on the activity is surprisingly small. Lengthening the ester group from methyl to butyl results in increased activity and decreased

aqueous solubility. The activity curves of these substances, however, often deviate from parallelism.

Influence on the Faecal Excretion of Wistar Rats

The influence of the substances, listed in Table II, on the number of faecal pellets passed by Wistar rats was investigated at the oral

Table II. Influence of oral doses of various substances on the faecal output of Wistar rats

Substance	mg/kg	Number of Treated rats	Average number of faecal pellets per group of 30 rats passed during the 3 days following oral dosage		
			1st day	2nd day	3rd day
solvent	—	600	1,106	1,160	1,189
R1132	1	90	809[a]	1,206	1,152
R1132	10	300	366[a]	646[a]	962[a]
R1132	100	60	87[a]	256[a]	617[a]
R1305	10	30	974	1,055	1,078
R1440	10	30	903	892	891
R1260	10	60	418[a]	707[a]	1,054
R1318	10	60	1,048	1,154	1,232
R1261	10	60	555[a]	873[a]	1,160
R1416	1	30	737[a]	1,195	1,240
R1416	10	60	318[a]	690[a]	966
R1319	1	30	746[a]	1,176	1,173
R1319	10	60	355[a]	635[a]	995[b]
R1355	1	30	720[a]	1,136	1,160
R1355	10	60	370[a]	730[a]	1,014
R1493	10	60	545[a]	792[a]	1,241
R1357	10	60	537[a]	931[b]	1,142
R1500	10	60	634[a]	919[b]	1,232
R1375	1	30	952	1,161	1,157
R1375	10	60	597[a]	960[b]	1,211
R1316	10	30	950	1,099	1,182
R1302	10	30	906[b]	1,107	1,200
R1320	10	30	901[b]	1,093	1,068
morphine	10	30	868[b]	1,179	1,223
morphine	100	30	403[a]	726[a]	1,064
atropine	10	30	615[a]	882[b]	1,124
codeine	10	30	674[a]	1,066	1,151

Probability of significant inhibitory effect of drug:
[a] $P > 0{\cdot}99$. [b] $P = 0{\cdot}99$–$0{\cdot}95$.

dose level of 10 mg/kg body weight. Among the 13 esters of the R 1132 series thus tested, 10 produced a significant decrease of faecal output during the first day after administration. The three inactive esters (R 1305, R 1318 and R 1440) as well as the ketones (R 1302, R 1316 and 1320), which are equally inactive in these experimental conditions were also found to be less active than the

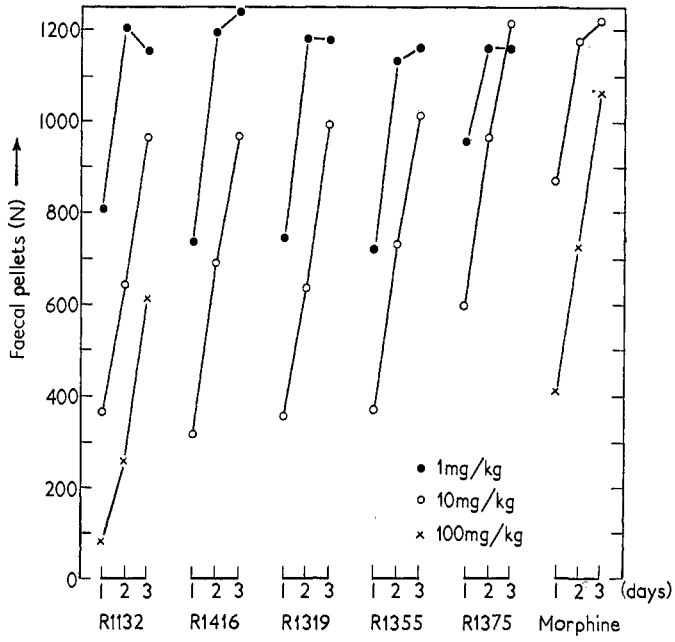


Fig. 2. The influence of oral doses of R 1132, R 1416, R 1319, R 1355, R 1375 and morphine on the number of faecal pellets passed by Wistar rats. (N = average number of faecal pellets passed by a group of 30 rats over a period of 24 h; days = days after administration.)

other members of the R 1132 series in the charcoal test in mice (Tables I and II).

Atropine and codeine are about equally active in rats, and more active than morphine which is only slightly active at the oral dose level of 10 mg/kg.

The ethyl-, *n*-propyl-, *n*-butyl- and *n*-amyl-esters (R 1132, R 1416, R 1319 and R 1355 respectively) were the most active compounds in rats and were selected, together with the less active

phenylethyl-ester R 1375 and morphine, for further study at the 1 and 100 mg/kg oral dose levels.

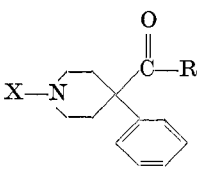
The results are graphically presented in Fig. 2. Obviously, the four alkyl esters are equiactive in all respects and about ten times more active than morphine; R 1375 is more active than morphine, but less active than the four other esters.

As compared with untreated rats, the daily faecal output of rats treated with oral doses of the 4 alkyl-esters may be summarized as follows:

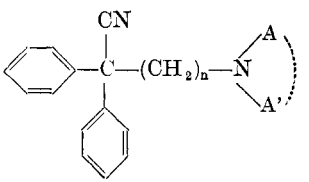
mg/kg	1st day	2nd day	3rd day
0	1,150±100	1,150±100	1,150±100
1	750± 50	1,150±100	1,150±100
10	350± 50	675± 50	975± 50
100	75± 50	250± 50	600± 50

Discussion

The R 1132 series is chemically related to pethidine (II: X = CH_3; R = OC_2H_5) and to the nitriles of the R 79 type (III).[7,8]



II



III

Lengthening of the ethyl group of pethidine results in a sharp decrease of analgesic activity.[9,10] This is also true for analgesically active esters of the pethidine type, where the N–CH_3–group is replaced by such groups as phenylethyl, cinnamyl, morpholinoethyl, etc.[10] As described above, however, the constipating activity of the esters of the R 1132 series (I) is largely independent of the actual length of the ester group. In compounds of type II the esters have greater analgesic activity than the ketones; likewise the esters of type I have greater constipating activity than the ketones.

The relation between chemical structure and pharmacological activity of the nitriles of the R 79 type (III) has been described in previous papers.[7,8,11] The lower tertiary dialkylamino bases as well as the tertiary heterocyclic amines (pyrrolidine, piperidine, etc.) of type III ($n = 2$) are typical atropine-like substances, whose potency is enhanced by quaternization. Lengthening of the side-chain (III; $n = 3$) results in decreased activity. All known amines of type III are devoid of significant analgesic activity. They are all less active than atropine in the charcoal meal test in mice and devoid of significant constipating activity in rats.[10] The members of the R 1132 series on the other hand are devoid of atropine-like properties. Lengthening of the side-chain ($n = 3$ or 4) results in decreased potency among the esters and in increased activity among the ketones of type I.

Summary. The R 1132 series is chemically related to the analgesics of the pethidine type and to the parasympatholytically active nitriles of the R 79 type. The compounds of the R 1132 series fail to produce significant analgesic or mydriatic activity after subcutaneous injection in mice and rats; they are, however, much more active in reducing the rate of gastro-intestinal propulsion and the rate of faecal output in these two species than any other known substance thus far tested in this laboratory.

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