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The pharmacology of 14-hydroxyazidomorphine

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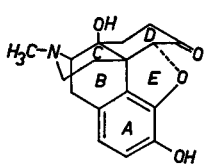
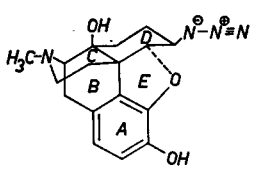
6-Deoxy-6-dihydroazido-14-hydroxyisomorphine (14-hydroxyazidomorphine) was synthesized, its analgesic potency in mice and rats, its antitussive effect in rats and its dependence liability in mice, rats and monkeys were studied. Azidomorphine, the 14-nonhydroxylated parent molecule, morphine, hydromorphone and oxymorphone were used for comparison. 14-Hydroxyazidomorphine proved to be as potent an analgesic as azidomorphine, even more potent as an antitussive, and showed the same low tolerance and dependence capacity. It was 11·6 times less toxic than azidomorphine in mice and 6·5 times less toxic in rats.

The introduction of a C_6-azido group into dihydroisomorphine increases analgesic activity and at the same time considerably decreases tolerance capacity and dependence liability relative to morphine (Knoll, Fürst & Kelemen, 1971). In animal experiments, 6-deoxy-6-dihydroazidoisomorphine (azidomorphine) proved to possess the highest analgesic effectiveness of the semisynthetic morphine derivatives (Knoll, 1973; Knoll, Fürst & Kelemen, 1973; Knoll, Fürst & Vizi, 1973). Azidomorphine is about 40–50 times more potent than morphine in the treatment of chronic intractable pain in man (Rétsagi & Schwarzmann, 1973), i.e. it is also the most effective semisynthetic morphine alkaloid in man.

It is well known that the introduction of a 14-hydroxy group into morphine decreases toxicity and, because the analgesic activity is usually increased, compounds with a better safety margin are produced (cf. Seki, 1965). With this in mind we synthesized 6-deoxy-6-dihydroazido-14-hydroxyisomorphine (14-hydroxyazidomorphine) and compared its pharmacological effects with the corresponding parent 14-nonsubstituted compound (azidomorphine) and with the structurally closest related



Oxymorphone



14-OH-azidomorphine

semisynthetic morphine alkaloids—hydromorphone and oxymorphone, previously considered the most potent of the semisynthetic analgesics in man (Eddy, Halbach & Braenden, 1956). The results are now presented.

METHODS AND MATERIALS

Toxicity

The test compounds as their bases were dissolved in 5% phosphoric acid and pH

of the solutions adjusted to 6 with N NaOH. Drugs were administered (5 ml kg^{-1}, s.c.) to Wistar rats, 120–150 g, and to mice, 18–22 g, of either sex. The LD50 was calculated according to Litchfield & Wilcoxon (1949).

Testing the analgesic effect

The hot plate test of Woolfe & McDonald (1944), modified by Pórszász & Herr (1950), the tail flick test of D'Amour & Smith (1941) and the writhing test of van der Wende (1956) modified by Witkin, Heuber & others (1961) and Koster & Anderson (1963) were used as described previously (Knoll & others, 1973). Each dose was tested on at least 10 rats and 20 mice on the hot plate, on 10 rats in the tail flick and on 15 mice in the writhing test. The ED50 was calculated on the basis of the dose-response curves.

Testing the antitussive effect

The method of Gösswald (1958) was used. Cough responses were elicited in rats by exposing them for 3 min to a citric acid aerosol (10%). The latency period between aerosol and onset of coughing was measured 18 h before and 30 min after the subcutaneous administration of the compounds. Only rats with less than 60 s control latency were used. The average control was found 29·4 $\pm$ 9·6 s.

Each dose was tested on at least 10 rats and the ED50 was calculated according to Litchfield & Wilcoxon (1949).

Testing of physical dependence

Mice. The 2 day test of Saelens, Granat & Sawyer (1972) was used.

Rats. The animals, 90–120 g, were injected subcutaneously twice daily (9 a.m. and 3.30 p.m.) with the test compound over 15 weeks then the abstinence syndrome was precipitated by the subcutaneous administration of 2·5 mg kg^{-1} naloxone hydrochloride instead of the morning dose of the analgesic. The development of physical dependence was assessed according to Buckett (1964).

Monkeys. Groups of six monkeys (*Rhesus macacus*), 7–8 kg, were injected subcutaneously morphine and 14-hydroxyazidomorphine, respectively, twice daily (9.30 a.m. and 4 p.m.) over 15 weeks, then the abstinence syndrome was precipitated by the subcutaneous administration of either 2·5 mg kg^{-1} nalorphine hydrochloride or 2·5 mg kg^{-1} naloxone hydrochloride instead of the morning dose of the analgesic. The development of physical dependence was assessed according to Seevers & Deneau (1963).

Drugs used. Morphine hydrochloride, nalorphine hydrochloride, hydromorphine base, oxymorphone base, azidomorphine base, 14-hydroxyazidomorphine base (alkaloids), naloxone hydrochloride (Endo Lab.).

RESULTS

Toxicity. 14-Hydroxylation of azidomorphine markedly reduced toxicity in mice and rats (Table 1). Azidomorphine was found to be 11·6 times more toxic in the mice and 6·5 times more toxic in the rats than 14-hydroxyazidomorphine by the subcutaneous route. The introduction of a 14-hydroxy group into hydromorphone reduced toxicity to a significantly lesser degree; hydromorphone was only about 2·3 times more toxic in the mice and 1·5 times more toxic in the rat than oxymorphone.

Table 1. *The acute toxicity in the mouse and rat of 14-hydroxyazidomorphine compared with azidomorphine, hydromorphone and oxymorphone.*

	Toxicity LD50 (mg kg^{-1})					
	Mouse			Rat		
Compound	i.v.	s.c.	oral	i.v.	s.c.	oral
Hydromorphone	69 (59–81)	120 (98–146)	280 (193–406)	—	110 (78–156)	—
Oxymorphone	82 (72–93)	280 (245–319)	490 (449–534)	95 (83–108)	160 (89–288)	140 (110–178)
Azidomorphine	13 (10–15)	16 (13–19)	58 (52–65)	8·1 (7·7–10·4)	13 (12–19)	62 (49–73)
14-Hydroxyazido-morphine	45 (32–60)	185 (135–253)	370 (322–436)	45 (31–65)	85 (75–96)	190 (113–253)

Values in brackets indicate 95% confidence limits.

Analgesic and antitussive effects

14-Hydroxylation of azidomorphine increased the antitussive potency (Table 3) but did not change significantly the analgesic effectiveness in the hot plate and writhing tests (Tables 2 and 3) and even decreased it in the tail flick test (Table 3). Oxymorphone, on the other hand, was found to be both as an analgesic and an antitussive more potent than hydromorphone, the 14-nonhydroxylated compound (Tables 2 and 3). The comparison of the LD50/ED50 ratios in Tables 2 and 3 clearly show that the 14-hydroxylated compounds have better safety margins than the nonhydroxylated ones and the difference is more pronounced with the 6-azido derivatives.

Table 2. *The analgesic activity in the mouse of* 14-*hydroxyazidomorphine compared with azidomorphine, hydromorphone and oxymorphone.*

Compound	Hot plate test ED50 mg kg^{-1}	Therapeutic index*	Writhing test ED50 mg kg^{-1}	Therapeutic index*
Hydromorphone	0·160 (0·146–0·174)	750	0·210 (0·165–0·266)	571
Oxymorphone	0·075 (0·060–0·086)	3733	0·122 (0·076–0·195)	2295
Azidomorphine	0·024 (0·012–0·045)	667	0·046 (0·038–0·054)	348
14-Hydroxyazido-morphine	0·029 (0·020–0·040)	6379	0·042 (0·035–0·049)	4405

* LD50/ED50 s.c.

Table 3. *The analgesic and antitussive activity in the rat of 14-hydroxyazidomorphine compared with azidomorphine, hydromorphone and oxymorphone.*

					Tail flick test		Antitussive test	
Compound	Hot plate test ED50 mg kg^{-1}			Therapeutic index*	ED50 mg kg^{-1} s.c.	Therapeutic index*	ED50 mg kg^{-1} s.c.	Therapeutic index*
	i.v.	s.c.	oral	s..c		s.c.		s.c.
Hydromorphone	0·170 (0·149–0·193)	0·220 (0·191–0·253)	23·0 (18·4–28·7)	500	0·220 (0·166–0·290)	500	0·26 (0·21–0·31)	423
Oxymorphone	0·065 (0·040–0·10)	0·080 (0·053–0·120)	13·5 (8·5–21·6)	2000	0·130 (0·087–0·192)	1231	0·058 (0·05–0·068)	2759
Azidomorphine	0·021 (0·011–0·037)	0·036 (0·019–0·066)	9·2 (6·0–12·9)	361	0·012 (0·0089–0·016)	1083	0·034 (0·027–0·042)	382
14-Hydroxyazido-morphine	0·021 (0·011–0·040)	0·036 (0·023–0·054)	16·0 (12·3–20·4)	2361	0·029 (0·022–0·037)	2931	0·021 (0·017–0·026)	4048

* LD50/ED50.

Tolerance and dependence. With azidomorphine we succeeded in demonstrating the possibility of a favourable dissociation between analgesic potency and physical dependence capacity (Knoll, 1973; Knoll & others, 1973).

Table 4 shows the dependence liability of 14-hydroxyazidomorphine compared with azidomorphine, morphine, hydromorphone and oxymorphone in mice. Considering the analgesic potencies, very small doses of morphine were administered in this series of experiments when compared to the doses of the semisynthetics studied. The effect of higher doses of morphine in this test was described earlier (cf. Knoll & others, 1973, Table 4). It is evident from Table 4 that the introduction of a 6-azido group into dihydroisomorphine apparently decreased the dependence capacity when compared to the structurally closest related semisynthetic morphine alkaloids which contain an oxo group at the 6-position. Azidomorphine, 5–6 times more potent as an analgesic than hydromorphine in mice (see Table 3), is less active in inducing naloxone-precipitated jumping. The same holds true for 14-hydroxyazidomorphine which is about three times more potent than oxymorphone in the hot plate and writhing tests (see Table 3) but much less effective in the mouse jumping test.

Table 4. *Physical dependence liability in the mouse of 14-hydroxyazidomorphine compared with azidomorphine, morphine, hydromorphone and oxymorphone* (2 day test results in the mouse jumping test).

		Total dose	Naloxone challenge (100 mg kg^{-1}, i.p.)	
Compound	Total dose mg kg^{-1}	Analgesic ED50	average jumps	jumped/ tested
Morphine	35	20	0·5	1/10
	70	40	6·1	5/10
	105	60	6·8	6/10
	210	120	19·9	8/10
Hydromorphone	35	233	24·9	8/10
	70	466	16·0	14/19
	105	700	49·1	9/10
Oxymorphone	35	573	45·3	10/10
	70	1147	30·0	14/15
	105	1721	37·7	10/10
Azidomorphine	35	1206	5·8	4/10
	70	2413	21·0	17/20
	105	3621	16·0	6/10
14-OH-azidomorphine	35	1346	9·6	4/10
	70	2692	9·0	15/20
	105	4038	19·7	6/7

Total dose was administered intraperitoneally in seven gradually increasing doses over 26 h. Hot plate test.

The same correlation was also found in rats. We earlier compared the development of tolerance to and dependence on morphine and azidomorphine in the rat (Knoll & others, 1973, Fig. 4). This time we tried to increase the daily dose of the azidomorphines during 15 weeks to higher levels and compared the effects with hydromorphone and oxymorphone.

Table 5 shows that up to the end of the experiments the analgesic effectiveness of daily doses of the azidomorphines were six times higher than that of the corresponding non-azides. The intensity of the naloxone-precipitated abstinence syndrome was only 2·6 with azidomorphine and 2·2 with 14-hydroxyazidomorphine, i.e. very low compared to 6·5, 6·0 and 6·8 with morphine, hydromorphone and oxymorphone,

Table 5. *Development of tolerance to and dependence on 14-hydroxyazidomorphine in the rat compared with azidomorphine, morphine, hydromorphone and oxymorphone.*

Compound	Analgesic ED50 mg kg^{-1} (hot plate) s.c.	Daily dose* n × ED50 1st day of treatment	85th day of treatment	Grade of tolerance (hot plate) DR**	Grade of dependence (scores)
Morphine	4·6	4	30	28·0	6·5
Hydromorphone	0·22	2	20	7·8	6·0
Oxymorphone	0·08	2	20	11·3	6·8
Azidomorphine	0·036	6	120	9·7	2·6
14-OH-azidomorphine	0·036	6	120	8·8	2·2

* Daily dose divided into two parts was injected subcutaneously at 9.30 a.m. and 3.30 p.m.
** Tested at the 14th week of treatment.

$$DR = \frac{\text{ED50 in chronically treated animals}}{\text{ED50 in controls}}$$

Abstinence syndrome was precipitated on 86th day of treatment by subcutaneous injection of 2·5 mg kg^{-1} naloxone hydrochloride instead of the morning dose of the analgesic. Scoring according to Buckett (1964).

respectively, and the level of tolerance reached was about the same. Thus the introduction of the azido group into isomorphine leads to a remarkable dissociation between analgesic potency and physical dependence liability.

Further support for this is given by Table 6, which compares the dependence liability of 14-hydroxyazidomorphine and morphine in the rhesus monkey. We reported previously (Knoll, 1973), that, when the analgesic potencies are considered, azidomorphine compared with morphine possesses a relatively low dependence capacity in the monkey. Now it has been possible to assess more quantitatively the difference between morphine and 14-hydroxyazidomorphine in their physical dependence capacities because the low toxicity of 14-hydroxyazidomorphine allowed us to increase the dose of this compound to very high levels. The dose of morphine (3 mg kg^{-1} subcutaneously, twice daily) was kept constant during the 15 weeks of experiments. This daily dose corresponded to only 1·5 times the analgesic ED50 on the hot plate when the drug was administered subcutaneously to rats (cf. Knoll

Table 6. *Comparison of the physical dependence liability of morphine and 14-hydroxyazidomorphine on monkeys (groups of 3).*

Compounds	Daily dose mg kg^{-1} at the beginning	last day before precipitation	Antagonist used for precipitation*	No. of animals experiencing intensity of abstinence syndrome** Moderate	Severe	Very severe
Morphine	3·0	3·0	Nalorphine HCl	–	1	2
	3·0	3·0	Naloxone HCl	–	–	3
14-Hydroxyazido-morphine	0·05	1·75	Nalorphine HCl	1	1	1
	0·05	1·75	Naloxone HCl	1	1	1

* Abstinence syndrome was precipitated on 86th day of treatment by subcutaneous injection of 2·5 mg kg^{-1} of the antagonist instead of the morning dose of the analgesic. Scoring according to Seevers & Deneau (1963).
** No animals had zero or mild intensity.

& others, 1973, Table 2). The dose of 14-hydroxyazidomorphine, however, was raised continuously and at the end of the 15th week we injected subcutaneously 1·75 mg kg^{-1}, twice daily, i.e. a dose which is 208 times the analgesic ED50 in the rat. Table 6 shows that the intensity of the abstinence syndrome precipitated by either nalorphine or naloxone was even to some extent higher in the morphine-treated monkeys. Thus, we had to reach much higher than 100-fold differences in the analgesic effectiveness to find doses of 14-hydroxyazidomorphine and morphine which could be considered as near equivalent in their dependence capacities.

DISCUSSION

14-Hydroxylation of azidomorphine brought about a favourable change in the pharmacological spectrum. 14-Hydroxyazidomorphine, when compared to the 14-non-hydroxylated parent compound, showed a much reduced toxicity, an equal analgesic and increased antitussive potency. Because after subcutaneous administration azidomorphine is 11·6 times more toxic in mice and 6·5 times more toxic in rats than 14-hydroxyazidomorphine, the latter seems to be more promising therapeutically.

The fact that 14-hydroxyazidomorphine was found to be as potent as azidomorphine as an analgesic, i.e. a further increase in the analgesic effectiveness was not attainable, might indicate that an upper limit had already been reached with the introduction of 6-azido group into dihydroisomorphine. Preliminary clinical studies show that in man too, 14-hydroxyazidomorphine, being about 40–50 times more potent than morphine, equals azidomorphine in its analgesic potency.

Azidomorphine showed a favourable dissociation between analgesic potency and dependence liability also in man. The dose of azidomorphine needed for total pain relief was administered to hundreds of patients with chronic intractable pain without the appearance of a significant grade of tolerance to or any sign of dependence on the drug (Rétsági & Schwartzmann, 1973). Nalorphine (10 mg kg^{-1}, i.v.) or naloxone (0·5 mg, i.v.) failed to precipitate the abstinence syndrome (the Himmelsbach system was used for scoring) in 12 patients treated over 2 to 11 weeks with daily doses of 1·55 to 3·75 mg azidomorphine, equivalent of 62–150 mg of morphine. Thus, in contrast, to the hitherto known semisynthetic morphine alkaloids, with azidomorphine we have attained a grade of dissociation between analgesic activity and dependence liability in man which seems to be of great importance.

The data presented show that like azidomorphine the 14-hydroxylated derivative also had a favourable dissociation between analgesic potency and dependence liability in mice, rats and monkeys.

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