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POSSIBLE MECHANISM OF THE LOW TOLERANCE CAPACITY OF AZIDOMORPHINE AND AZIDOCODEINE

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Abstract The *N*-demethylation by rat liver of azidomorphine and azidocodeine, new morphine derivatives with low tolerance and dependence capacity in animals and men, was compared to that of morphine. The drugs were administered intraperitoneally, in progressively increasing doses during a period of 27 days, until daily injections of 150 mg/kg of morphine HCl, 3 mg/kg of azidomorphine tartrate and 8 mg/kg of azidocodeine tartrate as maintenance doses were reached. The analgesic ratios of morphine:azidomorphine:azidocodeine were found to be 1:293:13 in the hot plate test. The liver of rats treated for 4 weeks with morphine completely failed to *N*-demethylate the narcotic drugs. The enzymic *N*-demethylation of morphine in animals exposed to more prolonged treatment (7 10 weeks) was about 30 40 per cent that of the saline treated controls. Withdrawal of morphine led to a complete restoration of the enzyme activity. In the azidomorphine- and azidocodeine-treated groups however, the *N*-demethylation capacity of the liver after 4 weeks treatment was only moderately reduced and in rats treated for 7 and 10 weeks, respectively, no decrease in the enzyme activity was observed. The profound difference between azidomorphine, the most potent known analgesic among the semisynthetic morphine derivatives, and morphine demethylation, parallels the difference in their tolerance capacity in animals and man.

IN PREVIOUS communications we described in detail the pharmacology of 6-desoxy-6-azidodihydroisomorphine (azidomorphine) and 6-desoxy-6-azidodihydroisocodeine (azidocodeine) which are newly synthesized morphine derivatives.[1-3] Azidomorphine, which was found to be about 300 times more potent than morphine in the rat (hot plate measurements) and 50 times more potent in man as an analgesic, proved to possess a very low tolerance capacity in mice and rats, and dependence on this drug failed to develop in rats and monkeys as well as in human beings.

Axelrod[4] demonstrated that in rats treated chronically with morphine the activity of the liver microsomal enzyme, which *N*-demethylates morphine, is profoundly reduced. He used this enzyme as a model for the morphine receptors, and the repression of the enzyme in morphine-treated rats led him to the conclusion that tolerance to the narcotic drugs may develop as a result of inactivation of receptor sites. Since in animals and man long-term treatment with azidomorphine or azidocodeine did not lead to the development of tolerance we compared the capacity of these compounds to that of morphine in repressing *N*-demethylase activity in the liver of the rat.

MATERIALS AND METHODS

Animal treatment. Male Sprague–Dawley derived (CFY strain) rats of 100 g body weight at the beginning of experiments were used. Morphine HCl, azidomorphine

TABLE 1. SOME PHARMACOLOGICAL DATA OF THE NARCOTIC DRUGS USED AND THE SCHEDULE OF TREATMENT

Drug	LD 50* (mg/kg)	ED 50‡ (mg/kg)	Relative potency	No. of animals treated	Schedule of treatment§ Initial dose (mg/kg)	Increment bidaily (mg/kg)	Maximum dose¶ (mg/kg)	No. of deceased animals‖
Morphine HCl	310† (267·2–349·6)	4·7 (2·9–7·8)	1	30	20	10	150	2
Azidomorphine tartrate	13 (11·7–19·0)	0·016 (0·007–0·038)	293	30	0·4	0·2	3	14
Azidocodeine tartrate	125 (100·0–150·2)	0·36 (0·16–0·79)	13	30	2	0·5	8	3

* Rat experiments; s.c. administration.
† Figures in parentheses indicate 95 per cent confidence limits.
‡ Hot plate test; rat experiments; s.c. administration.
§ Intraperitoneal administration.
¶ Reached on the 27th day of treatment.
‖ Number of animals deceased to the end of the 4th week of treatment.

tartrate and azidocodeine tartrate were injected intraperitoneally. The daily dose was increased every 2nd day, the maximum dose being reached by the 27th day of treatment and then maintained throughout the rest of the experiment. Control animals received daily injections of normal saline over the same period (see Table 1).

Enzyme assay. The animals were sacrificed 24 hr after the last injection, and their livers were removed immediately and chilled. Livers of at least two rats were pooled and a 30% homogenate was prepared in cold 0·1 M phosphate buffer pH 7·6 using a Potter-type of homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min at 0°. The supernatant containing microsomes and the soluble fraction was used.

Demethylation studies were carried out on treated and untreated rats on the same day in the same incubation medium. The use of daily controls always of the same age was important because of the age-dependent variation of enzyme activity.[5,6]

The incubation medium[7] contained 100 μmoles nicotinamide, 0·4 μmoles NAD, 0·4 μmoles NADP, 20 μmoles glucose-6-phosphate, 20 μmoles magnesium chloride, 70 μmoles semicarbazide, 6·5 μmoles substrate (in the case of azidocodeine 3·25 μmoles), 2·0 ml of liver supernatant (600 mg) and 0·1 M phosphate buffer pH 7·6, in a total vol of 6 ml. The mixture was incubated in air in a shaking apparatus at 37° for 1 hr. The reaction was stopped by the addition of 15 ml of 30% trichloroacetic acid. The formaldehyde formed was distilled[8] and the degree of enzymatic demethylation was determined by estimating the amount of HCOH by the chromotropic acid procedure.[9] Known concentrations of formaldehyde treated in the same way were used as standards. Correction was made by control incubations. The enzyme activity was expressed as μg HCOH/100 mg protein/hr. The protein content was determined by the method of Lowry *et al.*[10]

Special chemicals. 6-Desoxy-6-azidodihydroisomorphine tartrate (azidomorphine) and 6-desoxy-6-azidodihydroisocodeine tartrate (azidocodeine) synthetized by Bognár and Makleit[11] were obtained from Alkaloida Chemical Company, Tiszavasvári, Hungary; 1, 6-dimethyl-3-carbethoxy-4-oxo-6,7,8,9-tetrahydrohomopyrimidazole methylsulphate, synthesized by Mészáros *et al.*;[12] (Rymazolium, Probon, MZ-144) was obtained from Chinoin Pharmaceutical Works, Budapest, Hungary.

RESULTS

Figure 1 shows that in good agreement with the observation of Axelrod[4] daily intraperitoneal injections of morphine with progressively increasing doses led to a profound reduction of *N*-demethylating capacity of the liver tissue. The liver of rats treated for 4 weeks with morphine completely failed to *N*-demethylate this drug. The enzymic *N*-demethylation of morphine, however, was about 30–40 per cent that of the saline treated controls in animals exposed to a more prolonged treatment (7 and 10 weeks, respectively). Withdrawal of morphine for 2 weeks led to a complete restoration of the enzyme activity. In the morphine treated animals not only the *N*-demethylation of morphine but that of other substrates, like azidomorphine, azidocodeine, imipramine and Rymazolium[13–16] changed in the same manner.

Figure 2 demonstrates that in rats treated for 4 weeks with azidomorphine the *N*-demethylating capacity of the liver tissue was reduced much less than in the morphine-treated animals. This smaller reduction in enzymic demethylation was transient in nature. In animals treated for 7 and 10 weeks, respectively, a significant increase in the enzyme activity was observed. It is worth mentioning that the doses

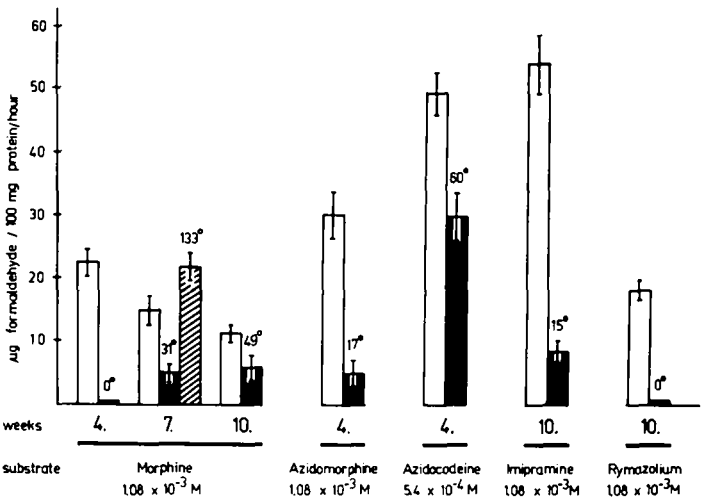

FIG. 1. The enzymic demethylation of various substrates by the liver microsomal supernatant of morphine hydrochloride-treated male Sprague Dawley derived (CFY) rats. (□) Rats treated with saline (controls). (■) Morphine-treated rats. Initial dose 20 mg/kg i.p. Daily dose increased every 2nd day until on the 27th day of treatment the maintenance dose of 150 mg kg was reached (▨) Treatment withdrawn for 2 weeks at the end of the 5th week. The enzyme activity expressed as a percentage of the control (number of experiments was five). The change in enzyme activity is statistically significant (○) $P < 0.05$, (●) $P < 0.01$.

of azidomorphine applied in these experiments were about five times more potent pharmacologically than those of morphine (compare data in Table 1). In good agreement with the low tolerance capacity of azidomorphine an increase in the dose of the drug was impossible. Table 1 shows that by the end of the 4th week, 14 out of 30 rats had succumbed in the azidomorphine-treated group.

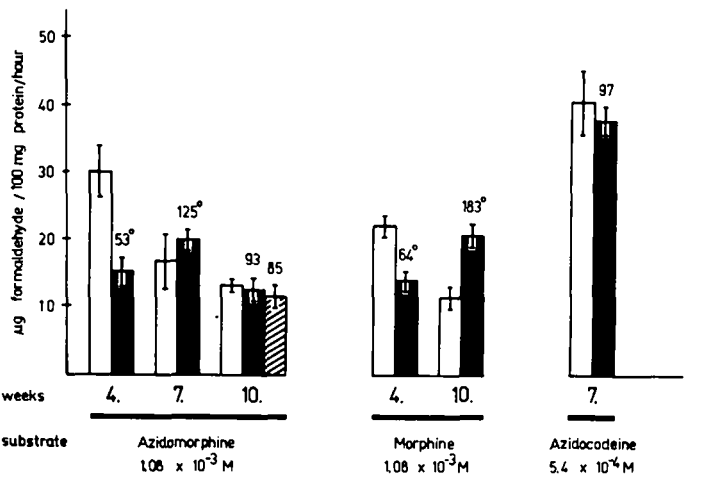

FIG. 2. The enzymic demethylation of various substrates by the liver microsomal supernatant of azidomorphine tartrate-treated male Sprague–Dawley derived (CFY) rats. (□) Rats treated with saline (controls). (■) Azidomorphine-treated rats. Initial dose 0·4 mg/kg i.p. Daily dose increased every 2nd day until on the 27th day of treatment the maintenance dose of 3 mg kg was reached. (▨) Treatment withdrawn for 2 weeks at the end of the 8th week. The enzyme activity expressed as a percentage of the control (number of experiments was five). The change in enzyme activity is statistically significant (○) $P < 0.05$, (●) $P < 0.01$.

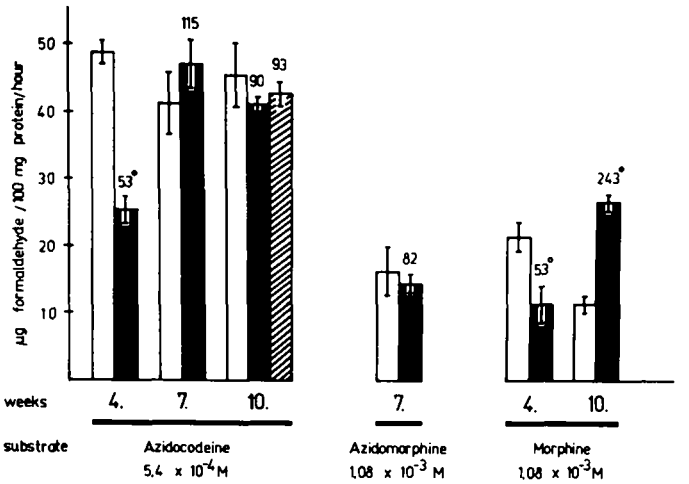

FIG. 3. The enzymic demethylation of various substrates by the liver microsomal supernatant of azidocodeine tartrate-treated male Sprague–Dawley derived (CFY) rats. (□) Rats treated with saline (controls). (■) Azidocodeine-treated rats. Initial dose 2 mg/kg i.p. Daily dose increased every 2nd day until on the 27th day of treatment and maintenance dose of 8 mg/kg was reached. (▨) Treatment withdrawn for 2 weeks at the end of the 8th week. The enzyme activity expressed as a percentage of the control (number of experiments was five). The change in enzyme activity is statistically significant (○) $P < 0.05$, (●) $P < 0.01$.

Figure 3 shows that in azidocodeine-treated animals the enzymic demethylation of morphine, azidomorphine and azidocodeine changed in the same manner as in the animals treated with azidomorphine.

DISCUSSION

The data presented in this paper agree with our earlier observations on the development of tolerance to the analgesic effect of azidomorphine in the rat.[1–3] We demonstrated that a transient state of low-grade tolerance to azidomorphine developed reaching its maximum at the 5th week of daily treatment with this compound. By the end of the 6th–7th week the sensitivity of the azidomorphine-treated animals to narcotic analgesics returned to the control level and was unchanged during further long-term treatment. Essentially the same type of changes were observed in azidomorphine-treated rats regarding the repression of the *N*-demethylase activity of the liver. These data favour the hypothesis that the liver enzyme which *N*-demethylates morphine possesses similarities to the receptors in the central nervous system which are selectively sensitive to morphine-like structures.[4,17,18]

Axelrod proposed[4] that the continuous interaction of morphine with its receptor inactivates the latter in due course. If we suppose that the C-6 alcohol group of morphine is involved in the chemical reaction leading to this "inactivation", it is reasonable to assume that the azido-group is inactive in this respect. According to this hypothesis we can look upon the azido-derivatives of morphine as a new type of morphine-relatives which maintain a high affinity for the receptor and possess intrinsic activity but are less reactive than morphine in "inactivating the receptor sites". This hypothesis corroborates the uncommon low tolerance and dependence capacity of azidomorphine which is the most potent semi-synthetic relative of morphine, hitherto known.

The mechanism of the repression of the *N*-demethylase system, and the chemical events leading to the supposed "inactivation of the receptor sites" are unknown. Several hypotheses have been forwarded to explain, on a biochemical basis, both tolerance and physical dependence[19–21] but further investigation is needed.[22] The azidomorphines may prove to be useful new tools in this field.

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