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Studies on the Metabolic N-Demethylation. VI.[1] Effect of Morphine and Nalorphine on the Oxidative Demethylation *in Vitro* in the Liver from Intact and Adrenalectomized Rats

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Administration of morphine and nalorphine decreased the activity of demethylating enzyme not only in intact rats but also in adrenalectomized rats. Therefore, it is considered that effects of morphine and nalorphine are not mediated through adrenal hormones.

On the other hand, single injection of nalorphine antagonized the drug metabolizing enzymes in rats which received a single injection of morphine, but when morphinization was continued, antagonism on the drug metabolizing enzymes of morphine and nalorphine disappeared.

In a previous work,[3] we have shown that the administration of morphine inhibits the N-demethylation of narcotic drugs, but does not affect the incorporation rate of ^{14}C-labeled amino acids into microsomal protein, while this incorporation apparently increases in the liver of phenobarbital treated rats.

Some workers[4-7] have suggested a certain relationship between decrease in the activity of enzyme which N-dealkylates narcotic drugs and the development of tolerance by a long-term administration of these drugs.

On the other hand, N-allylnormorphine, or nalorphine, is remarkable because when given by itself, it has many of the actions of morphine, but when given in conjunction with morphine, it antagonizes many of these actions.

It seemed of interest, therefore, to study the effect of nalorphine as a morphine antagonist on the demethylation reaction *in vitro*. In the present paper, effect of morphine and nalorphine on the oxidative demethylation in intact and adrenalectomized rats will be described.

The results of these experiments show that a single administration of morphine or nalorphine similarly inhibits the drug metabolism in both adrenalectomized and intact rats, and in morphine-treated rats, nalorphine shows reversal of the enzyme activity, *i.e.*, an antagonism to the effect of morphine on the oxidative demethylation.

Experimental

Materials——Morphine hydrochloride, nalorphine hydrochloride, meperidine hydrochloride, and sodium phenobarbital were commercially obtained.

Animals——Five each of male and female rats weighing 110—200 g were used. Animals were bilaterally adrenalectomized, maintained on 0.9% saline, and used 5 days after the operation.

Pretreatments with Drugs——Phenobarbital sodium was orally given in 1 ml of 0.9% saline in a dose

1) Part V: K. Minegishi, Y. Kuroiwa, and S. Okui, *Chem. Pharm. Bull.* (Tokyo), **16**, 1643 (1968).
2) Location: *Kita-4-bancho, Sendai.*
3) Y. Kuroiwa, K. Minegishi, and S. Okui, *Chem. Pharm. Bull.* (Tokyo), **13**, 731 (1965).
4) J. Axelrod and J. Cochin, *J. Pharmacol. Exptl. Therap.*, **121**, 107 (1957).
5) J. Axelrod and J. Cochin, *J. Phamacol. Exptl. Therap.*, **125**, 105 (1959).
6) J. Axelrod, *Science*, **124**, 263 (1956).
7) D.H. Clouet, and M. Rather, *J. Pharmacol. Exptl. Therap.*, **144**, 362 (1964).

of 500 μmoles/kg. Morphine hydrochloride and nalorphine hydrochloride dissolved in 0.5 ml of 0.9% saline were given intraperitoneally in a dose of 100 mg/kg.

Preparation and Assay of Enzyme Activity——Preparation of the enzyme was performed by the same method as reported previously.[1] The N-demethylation of drugs was measured by the formaldehyde produced in the reaction. Formaldehyde was established colorimetrically by the chromotropic acid method.[8]

Results

I. Effect of Morphine and Nalorphine on Oxidative Demethylation in the Liver of Intact Animals

Since meperidine was useful for measuring the degree of inhibition in the enzyme activity by narcotic drugs, as shown in the previous study,[3] meperidine was mainly used as a substrate in the present work.

Table I shows that the activity of oxidative demethylating enzyme was similarly decreased by the administration of the same dosage of morphine or nalorphine. The administration of drugs in a dose of 80—100 mg/kg produced a marked inhibition.

Table I. Effect of Morphine and Nalorphine on Oxidative Demethylation in the Liver of Intact Animals

Dose (mg/kg, *i.p.*)	Pretreatment with	
	Morphine	Nalorphine
Control	4.23±0.36[a]	4.23±0.36[a]
20	3.71±0.37	3.39±0.31
40	2.87±0.17	3.19±0.59
80	2.71±0.44	2.35±0.18
100	2.30±0.32	2.26±0.47
200	2.49±0.25	2.43±0.10

substrate: meperidine
a) unit: formaldehyde μmoles/hr/g liver
Values are expressed as mean ± standard deviation.

II. Time Course of the Enzyme Activity in Liver from Nalorphine-treated Rats

As seen in Table II, the enzyme activity was gradually reduced from 4 to 20 hr after pretreatment with nalorphine. This inhibitory effect of nalorphine on the demethylation reaction is similar to that of morphine 24 hr after the pretreatment.

Table II. Time Course of the Enzyme Activity in Nalorphine-treated Rats

Hours after nalorphine injection	Demethylating activity	Hours after nalorphine injection	Demethylating activity
Control	4.23±0.36[a]	16	2.52±0.35
4	3.15±0.30	20	2.15±0.42
8	2.05±0.53	24	2.26±0.31
12	3.13±0.32		

substrate: meperidine
a) uint: formaldehyde μmoles/hr/g liver (mean ± S.D.)
Nalorphine hydrochloride was given intraperitoneally in a dose of 100 mg/kg. Animals were killed 4, 8, 12, 16, 20, and 24 hours after the injection.

8) R.M. Burton, *Methods in Enzymology*, 3, 247 (1957).

III. Antagonism of Morphine by Nalorphine on the Activity of Drug-metabolizing Enzymes

Given in conjunction with morphine, nalorphine acts as an antagonist of the action of morphine. This effect can be seen in the level of drug metabolizing enzyme as shown in Fig. 1, that is, the enzyme activity was decreased to 54% of the control activity by the administration of morphine (100 mg/kg) alone, but the administration of nalorphine ranging from 20 mg to 100 mg/kg considerably recovered the enzyme activity in morphine-treated rats. However, the administration of excess dose (200 mg/kg) of nalorphine produced no antagonism.

Table III indicates that the antagonism mentioned above was efficiently observed when nalorphine was given one hr after pretreatment with morphine.

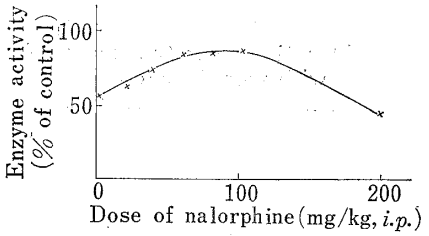

Fig. 1. Effect of Various Doses Nalorphine as a Morphine Antagonist on the Activiy of Drug-metabolizing Enzymes

Nalorphine was intraperitoneally administered in doses of 20, 40, 60, 80, 100, or 200 mg/kg, one hour after *i.p.* administration of 100 mg/kg of morphine. Animals were killed 24 hours after the last injection.

Table III. Effect of Nalorphine as a Morphine Antagonist on the Oxidative Demethylation by the Difference of Administration Time

Pretreatment with	Activity	Ratio (%)
None	2.54±0.14[a)]	100
Morphine	1.07±0.10	42.1
Morphine + nalorphine (before 1 hr)	1.20±0.08	47.2
Morphine + nalorphine (at the same time)	1.84±0.06	72.8
Morphine + nalorphine (after 1 hr)	1.98±0.05	87

substrate: meperidine
a) unit: formaldehyde μmoles/hr/g liver (mean ± S.D.)
Morphine and nalorphine hydrochloride were given by *i.p.* injection in doses of 100 and 60 mg/kg respectively. Animals were killed 24 hours after the last injection.

IV. Effect of Nalorphine and Phenobarbital on the Oxidative Demethylation in Liver from Morphinized Rats

The effect of nalorphine on the oxidative demethylation in the liver from morphinized rats is shown in Table IV. The second line in this Table shows that the activity of demethylating enzyme was decreased to 59% of the control by the administration of nalorphine alone but, as shown in the fourth line of this Table, the reduced activity caused by pretreatment with morphine was marked by recovered by the administration of nalorphine.

It is found that the effect of nalorphine as a morphine antagonist can be observed on the activity of demethylating enzyme in the liver of rat pretreated with morphine just one day but not more than two days later.

Examination was made to see whether the synergistic effect of nalorphine and phenobarbital will be obtained or not on the oxidative demethylation in morphinized rats. Synergistic effect of these drugs did not appear, as shown in Table V, and the activity of demethylating enzyme gradually diminished without any promotion by both drugs in rats in which morphinization was progressing.

V. Effect of Morphine and Nalorphine on the Oxidative Demethylation in Liver of Adrenalectomized Male Rats

Table VI shows the effect of morphine and nalorphine on the oxidative demethylation in the liver from adrenalectomized rats.

The effect of operation could scarcely be seen in sham-operated rats, but the activity of demethylating enzyme was markedly decreased by adrenalectomy. Furthermore, the

Table IV. Effect of Nalorphine on the Oxidative Demethylation in Morphine Treated Rats

Duration of pretreatment with morphine (days)	Administration of nalorphine	Demethylating activity	Ratio (%)
0	0	4.54±0.27[a]	100
0	+	2.68±0.35	59.0
1	0	2.31±0.14	50.9
1	+	4.37±0.13	96.3
2	0	2.10±0.11	46.3
2	+	2.63±0.34	57.9
4	0	2.04±0.13	44.9
4	+	2.29±0.13	50.4
8	0	1.70±0.13	37.4
8	+	1.56±0.41	34.4

substrate: meperidine
a) unit: formed formaldehyde μmoles/hr/g liver (mean ± S.D.)
Animals were treated with drugs as follows: Nalorphine hydrochloride was given only once by the *i.p.* injection in a dose of 100 mg/kg 1 hour after the last administration of morphine (100 mg/kg). Animals were killed 24 hours after the injection of morphine.

Table V. Effect of Nalorphine and Phenobarbital on Oxdiative Demethylation in Morphine-treated Rat Liver

Pretreatment with morphine (days)	Aministration of		Demethylating activity	Ratio (%)
	Nalorphine	Phenobarbital		
0	0	0	4.54±0.27[a]	100
0	+	+	3.74±0.16	82.4
1	+	+	4.99	109.9
2	+	+	3.55±0.38	78.2
4	+	+	2.39±0.09	52.6
8	+	+	1.54±0.41	33.9

substrate: meperidine
a) unit: formed formaldehyde μmoles/hr/g liver(mean ± S.D.)
Doses and method of administration of morphine and nalorphine were the same as described in Table IV. Phenobarbital was given orally in a dose of 500 μmoles/kg at the same time when nalorphine was injected.

activity of demethylating enzyme in the liver from adrenalectomized rats pretreated with morphine or nalorphine was lower than that of adrenalectomized control rats.

The effect of nalorphine as morphine antagonist was similarly observed on the oxidative demethylation in adrenalectomized rats as well as in intact animals.

VI. Effect of Morphine and Nalorphine on the Oxidative Demethylation in the Liver of Adrenalectomized Female Rats

As shown in Table VII, it was also observed in female rats that the activity of demethylating enzymes was decreased by the administration of morphine or nalorphine, but the inhibitory effect in female rats is less significant than that in male rats, especially in the case of nalorphine.

The effect of morphine or nalorphine in adrenalectomized female rats is similar to that in intact rats, but the antagonism of morphine by nalorphine on the oxidative demethylation was not observed in adrenalectomized female rats.

Table VI. Effect of Morphine and Nalorphine on the Oxidative Demethylation in the Liver of Adrenalectomized Male Rats

Animals	Pretreatment with drug	Activity	Ratio (%)
Control	none	3.58±0.09[a]	100
Sham-operated	none	3.37±0.25	94.1
Adrenalectomized	none	2.33±0.06	65.0
Adrenalectomized	morphine (20 mg/kg)	1.38±0.38	38.5
Adrenalectomized	nalorphine (20 mg/kg)	2.19±0.14	61.2
Adrenalectomized	morphine (50 mg/kg)[b]	0.57	15.9
Adrenalectomized	nalorphine (50 mg/kg)	1.63±0.24	45.5
Adrenalectomized	morphine–nalorphine[c] (20 mg/kg resp.)	2.56±0.37	71.5

substrate: meperidine
a) unit: formaldehyde μmoles/hr/g liver (mean ± S.D.)
b) Several animals died by the administration of morphine in a dose of 50 mg/kg.
c) Nalorphine was given 1 hour after the administration of morphine.

Table VII. Effect of Morphine and Nalorphine on the Oxidative Demethylation in the Liver of Adrenalectomized Female Rats

Animals	Pretreatment with	Activity	Ratio (%)
Intact rats[b]	none	2.12±0.36[a]	100
	morphine	1.51±0.29	71.2
	nalorphine	1.70±0.33	80.2
	morphine + nalorphine	1.92±0.11	90.6
Adrenalectomized rats[b]	none	1.27±0.37	100
	morphine	0.68±0.22	53.5
	nalorphine	1.02±0.25	80.3
	morphine + nalorphine	0.75±0.30	59.2

substrate: meperidine
a) unit: formaldehyde μmoles/hr/g liver (mean ± S.D.)
b) Dose of morphine and nalorphine were 100 mg/kg *i.p.* for intact rats and 20 mg/kg for adrenalectomized rats. Female rats weighing 90—120 g were used in this experiment.

Discussion

The most striking property of nalorphine is its marked ability to promptly abolish many of the actions of morphine in mammals, although when given by itself, it has an action similar to morphine.

As shown in this investigation, nalorphine does inhibit N-demethylation of meperidine as well as of morphine in liver microsomal preparation from intact and adrenalectomized rats (Tables I, II, and VI) but the inhibitory effect of morphine for the demethylation reaction is antagonized by the administration of nalorphine.

The degree of antagonism is related to the dose of nalorphine; a dose of 20—100 mg /kg significantly reverses depression of the enzyme activity produced by 100 mg/kg of morphine. However, a high dose (200 mg/kg) of nalorphine does not reverse the reduced activity of the enzymes in the liver from rats pretreated with morphine (Fig. 1).

Since this effect of antagonism cannot be recognized when morphinization is in progress, it is considered that nalorphine is a pharmacological, rather than a chemical, antagonist.

Woods, *et al.* reported the plasma level of nalorphine in dogs[9] and that of morphine in cats.[10] From their data, nalorphine seems to be excreted faster than morphine. These

9) L.A. Woods and H.E. Muehelenbeck, *J. Pharmacol. Exptl. Therap.*, **120**, 52 (1957).
10) H.I. Chernov and L.A. Woods, *J. Pharmacol. Exptl. Therap.*, **149**, 146 (1965).

data agree with our experiments as shown in Table III, that is, antagonism of nalorphine against morphine is most effective on the activity of demethylating enzymes when nalorphine is given after pretreatment with morphine.

As mentioned above, low activity of the drug metabolizing enzyme by a long-term administration of morphine is not reversed by pretreatment with nalorphine and not activated by phenobarbital (Table V). Although these results cannot be accounted for by the simple conception of competitive inhibition of or adaptation to the drugs, it is conceivable that the ability to detoxicate drugs in the liver may be destroyed pathologically by the chronic toxicity of morphine. This consideration will be supported[11] from the data indicating amount of morphine glucuronide to decrease gradually by a long-term administration of morphine. Takemori[12] also pointed out that the activity of glucuronyl transferase in the animal liver is decreased by an extended morphinization.

On the other hand, many attempts have been made to correlate the function of adrenal cortex to the activity of several enzymes and drug response.

After rats were pretreated for several days with morphine, metabolism of hexobarbital in liver slice was inhibited. This effect of morphine on the metabolism of hexobarbital was reversed by prior administration of ACTH.[13]

Our data showed that adrenalectomized rats have a lower resistance than intact rats against morphine, and the activity of demethylating enzymes is also reduced in the liver of adrenalectomized rats. There might, therefore, be some regulating systems mediated through adrenal hormone in a part of that enzyme system but, as shown in Table VI, it is likely that the effects of morphine and nalorphine are not directly mediated through the adrenal.

Some workers[14,15] have reported that there are sex differences in drug metabolism in rats but not in other mammals. It is generally accepted that the activities of drug metabolizing enzyme in male rats are higher than those in females. March and Elliott[16] reported that male rats demethylate morphine more rapidly than females, and in tracer experiments, exhale more $^{14}CO_2$. This is one of a number of experimental findings which suggest that the anabolic effect of androgen produces a sex difference in the amount of enzymes available for some biochemical processes.

In the present investigation, the inhibitory effect of morphine and nalorphine on the demethylation appeared similarly in intact male and female rats, but the effect of these drugs in male rats is stronger than in females. There is also a sex difference in the effect of nalorphine as a morphine antagonist which was clear in adrenalectomized male rats but not in adrenalectomized female rats (Table VI and VII).

On the basis of these studies, it appears that nalorphine can antagonize the action of morphine on the level of drug metabolizing enzyme system and that the effects of morphine and nalorphine are not directly mediated through the adrenal.

11) S. Okui, *Kosei Kagaku Kenkyu Hokoku*, 33 (1962).
12) A.E. Takemori, *J. Pharmacol. Exptl. Therap.*, 130, 370 (1960).
13) W.F. Bousquet, R.R. Howell, and T.S. Miya, *Biochem. Pharmacol.*, 13, 123 (1964).
14) G.P. Quinn, J. Axelrod, and B.B. Brodie, *Biochem. Pharmacol.*, 1, 125 (1958).
15) R. Kato, E. Chiesara, and G. Frontino, *Biochem. Pharmacol.*, 11, 221 (1962).
16) C.H. March and H.W. Elliott, *Proc. Soc. Exptl. Biol. Med.*, 86, 494 (1954).