— SHORT COMMUNICATIONS

EFFECT OF MORPHINE ADMINISTRATION ON THE ACTIVITIES OF
MICROSOMAL DRUG-METABOLIZING ENZYME SYSTEMS
IN LIVER OF DIFFERENT SPECIES

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Axelrod reported that repeated injection of morphine to male rats markedly depressed the activity of N-demethylation of morphine. He suggested the similarity in the development of tolerance and depression of the activity of N-demethylation of morphine in liver microsomes (1, 2). However, Herken *et al.* demonstrated that the administration of morphine decreased not only N-demethylation of the narcotics having cross tolerance to morphine, but it also decrease N-demethylation of aminopyrine and hydroxylation of hexobarbital (3). Moreover, Kato and Gillette observed that a single injection of morphine to male rats decreased N-demethylation of morphine and aminopyrine and hydroxylation of hexobarbital, in contrast, same single injection of morphine to female rats did not decrease the activities of these enzyme systems (4, 5). Furthermore, they also showed that the administration of morphine to both sex of rats did not decrease the activities of enzyme systems having no clear sex difference, such as hydroxylation of aniline (4, 5).

The activities of N-demethylation of aminopyrine and hydroxylation of hexobarbital in castrated male rats did not show further decrease by the administration of morphine, however, the activities of these enzyme systems in female rats, previously castrated and treated with methyltestosterone, were markedly depressed like as in male rats.

Thus, Kato and Gillette (5) and moreover Kato and Kitagawa (6) proposed a new interpretation that the depressive action of morphine on the activities of drug-metabolizing enzyme systems of rat liver microsomes is not related to the development of the tolerance, but it acts, probably indirectly, on the enzyme systems which is stimulated by male sex hormone.

On the other hand, Brodie, and Kato *et al.* reported that the sex difference in the metabolism of hexobarbital (7), carisoprodol (8, 9) and strychnine (9, 10) by liver microsomes was only observed in rats, but not observed in other species of animals and even so the administration of high dose of methyltestosterone or 4-chlortestosterone did not increase the enzyme activities. Thus, if the interpretation proposed by us would be correct, the depressive action of morphine on the activities of drug-metabolizing enzyme systems of liver microsomes should be limited on rats.

The results of present communication are in accord with this view. Both sex of adult rats, mice, guinea-pigs and rabbits were used. The method for the determination of various enzyme activities were almost same as reported in a previous paper (5). Determination of aminopyrine content in serum was carried out according to the method of Brodie *et al.* with a slight modification (11).

TABLE 1. Effect of morphine administration on the activities of drug-metabolizing enzyme systems of liver microsomes of different species of animals.

Species	Sex	Morphine treatment	Aminopyrine N-demethylation (m μ mole/30 min/g)	Morphine N-demethylation (m μ mole/30 min/g)	Hexobarbital hydroxylation (m μ mole/30 min/g)	Aniline hydroxylation (m μ mole/30 min/g)
Rats	Male	—	428 $\pm$ 21	1059 $\pm$ 38	2027 $\pm$ 92	404 $\pm$ 24
"	"	+	195 $\pm$ 18 (-54)	603 $\pm$ 40 (-43)	992 $\pm$ 73 (-51)	368 $\pm$ 28 (- 9)
"	Female	—	117 $\pm$ 13	382 $\pm$ 28	629 $\pm$ 41	353 $\pm$ 20
"	"	+	109 $\pm$ 18 (- 7)	360 $\pm$ 31 (- 6)	601 $\pm$ 45 (- 5)	341 $\pm$ 25 (- 3)
Mice	Male	—	585 $\pm$ 28	841 $\pm$ 35	1921 $\pm$ 75	913 $\pm$ 20
"	"	+	553 $\pm$ 34 (- 6)	809 $\pm$ 33 (- 4)	1870 $\pm$ 89 (- 1)	908 $\pm$ 25 (- 1)
"	Female	—	563 $\pm$ 19	803 $\pm$ 31	1843 $\pm$ 62	925 $\pm$ 27
"	"	+	572 $\pm$ 28 (+ 1)	815 $\pm$ 45 (+ 1)	1752 $\pm$ 73 (- 5)	883 $\pm$ 21 (- 5)
Guinea-pigs	Male	—	318 $\pm$ 23	415 $\pm$ 39	811 $\pm$ 39	415 $\pm$ 38
"	"	+	301 $\pm$ 30 (- 5)	423 $\pm$ 41 (+ 2)	852 $\pm$ 52 (+ 5)	429 $\pm$ 43 (+ 3)
"	Female	—	321 $\pm$ 26	403 $\pm$ 43	852 $\pm$ 55	427 $\pm$ 35
"	"	+	298 $\pm$ 28 (- 7)	391 $\pm$ 34 (- 3)	802 $\pm$ 34 (- 6)	411 $\pm$ 45 (- 4)
Rabbits	Male	—	240 $\pm$ 28	705 $\pm$ 55	1008 $\pm$ 78	718 $\pm$ 83
"	"	+	235 $\pm$ 23 (- 2)	721 $\pm$ 47 (+ 2)	1002 $\pm$ 90 (- 1)	752 $\pm$ 56 (+ 5)
"	Female	—	225 $\pm$ 22	711 $\pm$ 61	1072 $\pm$ 72	703 $\pm$ 54
"	"	+	243 $\pm$ 31 (+ 8)	684 $\pm$ 70 (- 4)	993 $\pm$ 105 (- 7)	670 $\pm$ 88 (- 5)

Both sex of adult rats, mice, guinea-pigs and rabbits were treated with morphine 30 mg/kg, 150 mg/kg, 50 mg/kg and 50 mg/kg (s.c.) respectively. The numbers of animal used were 48 rats, 120 mice, 24 guinea-pigs and 20 rabbits. All animals were killed 36 hours after the morphine administration. Pooled livers from 2 rats and 5 mice were used for the assay of enzyme activities. The enzyme activities are given by amount of metabolized drugs for 30 minutes per gr. wet liver and given as averages $\pm$ standard errors from 5-6 determinations. The numerals in the brackets indicate the difference in per cent from non-treated animals.

FIG. 1. Effect of single injection of morphine on the *in vivo* metabolism of aminopyrine in male rats. Male rats were treated with morphine (30 mg/kg, s.c.) 36 hours before the administration of aminopyrine (150 mg/kg, i.p.). Each groups of rats were consisted in 6 rats and killed 15 minutes, 30 minutes, 60 minutes, 120 minutes and 180 minutes later respectively. The calculated metabolism of aminopyrine for 1 hour are 56% and 35% respectively for control and morphine treated rats.

B.H.L.=Biological half life.

interpretation that the administration of morphine blocks the action of male sex hormone to stimulate the activities of some microsomal enzyme systems of rat liver.

It is, therefore, reasonable that the activities of aniline hydroxylation in male rats, and the activities of aminopyrine N-demethylation, morphine N-demethylation, hexobarbital hydroxylation and aniline hydroxylation in female rats were not significantly affected by the administration of morphine.

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Table 1 shows that the activities of aminopyrine N-demethylation, morphine N-demethylation and hexobarbital hydroxylation of liver microsomes from male rats are markedly higher than that from female rats, in contrast, there are no such differences in liver microsomes from mice, guinea-pigs and rabbits. Moreover, depressive effect of morphine on the activities of the microsomal enzymes were observed only in male rats, but not in male mice, guinea-pigs and rabbits. The activity of aniline hydroxylation, which have no clear sex difference in rats, was not depressed in male rats as well as male mice, guinea-pigs and rabbits.

All the previously reported experiments of the morphine effect on the activity of liver microsomal enzymes was carried out *in vitro* levels, thus, there was possibility that the liver microsomal enzymes of the male rats treated with morphine are unstable during the preparation of the microsomes and so lost their activities.

Fig. 1 shows that the single injection of morphine to male rats decreases the metabolism of aminopyrine as well as *in vitro* experiments. These results were in accord with our proposed