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ENHANCED METABOLISM OF MORPHINE IN *OCTODON DEGUS* COMPARED TO WISTAR RATS

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Abstract—1. Comparative studies of the actions of morphine in different mammals have shown that *Octodon degus* presents an unusual tolerance to this compound.

2. Morphine glucuronidation and N-demethylation in microsomal fractions of *Octodon degus* were 10.9 and 50.0 nmol of product formed/min/g of wet liver, respectively. In Wistar rat these activities were 10.5 and 12.5, respectively.

3. Microsomal protein and cytochrome *P*-450 contents were two and four times higher in *Octodon degus* than in the Wistar rat, respectively.

4. These results may explain the high tolerance to morphine presented by *Octodon degus*.

INTRODUCTION

Previous studies of the effects of morphine on the central nervous system of small mammals have shown that *Octodon degus*, a chilean caviomorph, presents a natural resistance to this compound. In fact, this rodent did not display analgesia as measured by the hot plate method and exhibited no central depression (catatonia) after high i.p. doses of morphine (Villanueva *et al.*, 1980). After high doses of morphine only 50% of *Octodon degus* exhibited a slight increase of voltage at electroencephalogram, whereas all the rats displayed a high increase of voltage with lower doses of morphine (Villanueva *et al.*, 1980). Also, the antagonistic effect of high doses of morphine on the induced intestinal contractions by augmenting the intraluminal tension *in situ* was only observed in guinea-pig but not in *Octodon degus* (Villanueva *et al.*, 1980).

It was not established whether this natural tolerance of *Octodon degus* is due to the existence of a reduced number of pharmacological receptors or to differences in drug metabolizing enzymes.

In order to assess the nature of this resistance, comparative studies on morphine disposition in Wistar rats and *Octodon degus* were undertaken. Studies on the oxidative metabolic pathways and glucuronide reactions were also performed.

METHODS

Animals and chemicals

Groups of male Wistar rats and male *Octodon degus* of 200–220 g, inbred in our animal room were used throughout these studies. Animals were fed with Purina laboratory chow and water *ad libitum*. Morphine sulfate was purchased form Mallinckrodt Chemical Company (St Louis, Mo.) and [N-methyl-^{14}C]morphine hydrochloride with a sp. act. of 57 mCi/mmol was obtained from Amersham Searle (Arlington Heights; Ill; U.S.A.). The radiochemical purity of [^{14}C]morphine was more than 98% when checked by thin layer chromatography. Uridine diphosphoglucuronic acid (ammonium salt), β-glucuronidase (type B_{10}), bovine serum albumin, glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from Sigma Chemical Company (St Louis, MO). Animals were injected subcutaneously with a freshly prepared solution of morphine sulfate and morphine hydrochloride 7.5 mg/10 μCi/kg of body wt, for tissue distribution studies. The injection volume was 2.0 ml/kg body wt.

Tissue distribution of [N-methyl-^{14}C]morphine

Concentrations of total radioactivity of unchanged soluble metabolites in blood, brain, liver and kidney were measured at several time interval after administration of [^{14}C]morphine to Wistar rats and *Octodon degus*. Groups of three Wistar rats and *Octodon degus* were killed by decapitation at 30, 60 and 120 min after s.c. injection of morphine sulfate (7.5 mg/kg body wt) and [N-methyl-^{14}C]morphine hydrochloride (10 μCi/kg body wt). Blood samples were collected in heparin treated tubes after decapitation of the animals. Brains, kidneys and livers were immediately removed, weighed and frozen until analyzed.

Total [^{14}C]morphine determination in tissues

[^{14}C]morphine tissue distribution studies were determined by measuring the radioactivity contained in 0.5 ml of 20% w/v tissue homogenates as described by Liu and Wang (1975). Corrections for quenching were performed by adding known amounts of [^{14}C]morphine hydrochloride (2000–10,000 cpm) per gram of tissue homogenates.

Determinations of unchanged morphine and its polar metabolites

The amounts of unchanged morphine and the polar metabolites in kidneys and livers were performed by the method of Yeh and Woods (1972). Two milliliters of 20% (w/v) tissue homogenates were adjusted at pH 9.7 with 40% K_2HPO_4 and extracted three times with 10 ml of a solvent mixture of ethylen dichloride: n-amyl alcohol (70:30). The organic phases of each sample were carefully aspirated and pooled. Four milliliter aliquots of the organic phase were added to 15 ml of Aquasol and the radioactivity was counted in a liquid scintillation counter. The radioactivity present in the aqueous phase was determined in vials containing 1.0 ml aliquots, 6.0 ml water and 10 ml Aquasol. After vigorous shaking to form a clear gel, the samples were counted in a liquid scintillation counter. The nature of ^{14}C

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polar metabolites separated from the aqueous phase was determined essentially as described by Liu *et al.* (1975) by using thin layer chromatography and hydrolizing the aqueous phases with 2000 units of β-glucuronidase at pH 5.0 followed by the free morphine extraction as described before. Over 87% of water polar metabolites corresponded to morphine glucuronide.

Unreacted morphine and its polar metabolites in blood were determined by extracting 0.5 ml of blood (at pH 9.7) with the solvent mixture, ethylene dichloride: n-amyl alcohol (70:30) following the procedure described for tissue analysis. All the determinations were performed in duplicate.

In vitro *metabolism*

UDP-glucuronidation and N-demethylation of morphine were studied using liver microsomes from both Wistar rat and *Octodon degus*. After 18 hr of fasting, four animals from each group were decapitated and the livers were perfused with ice cold 0.9% NaCl. Liver microsomes from pooled livers of each group of animals were prepared as described previously by Baron and Tephly (1970). Microsomal protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Cytochrome *P*-450 content was determined by the different spectrum induced by carbon monoxide as described by Omura and Sato (1964a, b). The reaction mixture for morphine glucuronide synthesis contained: [N-methyl ^{14}C]morphine hydrochloride in variable concentrations from 0.2 to 2.0 mM (sp. act.: 600 dpm/nmol); 5.0 mM UDP-glucuronic acid; 50 mM Tris-HCl, pH 8.0; 5.0 mM $MgCl_2$; and 1.0 mg of microsomal protein in a total volume of 0.5 ml. Control samples had no UDP-glucuronide acid. Reaction mixtures were incubated for 20 min at 37°C. The reaction was stopped by addition of 1.5 ml of 4.5% trichloroacetic acid. The estimation of morphine glucuronide was performed by scintillation counting technique according to the method described by del Villar *et al.* (1974). The reaction mixture for the assay of morphine N-demethylation contained: variable concentrations of [N-methyl ^{14}C]morphine hydrochloride from 0.25 to 2.0 mM (sp. act.: 600 dpm/nmol); 35 mM Tris HCl, pH 8.0; 3.5 mM $MgCl_2$; 0.1 M glucose-6-phosphate; 5 Kornberg Units glucose-6-phospahte dehydrogenase and 1.5 mg of microsomal protein in a total volume of 1.0 ml. Control samples had no glucose-6-phosphate dehydrogenase. Reaction mixtures were incubated for 20 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 10% trichloroacetic acid. The estimation of [^{14}C]formaldehyde formed in the assay of morphine N-demethylation was performed by scintillation counting technique according to the method described in Sánchez *et al.* (1982). Reaction rates were determined at conditions where product formation was linear with time and protein concentration.

Statistical analysis

Student's test was used to determine the significance of differences between Wistar rats and *Octodon degus*; $P = 0.05$ was the minimal level of significance.

RESULTS

Distribution of morphine

Blood levels of total morphine are shown in Table 1. No significant differences were observed in morphine concentrations between both rodents at 30 and 60 min after morphine administration. However, at 120 min the level was higher in Wistar rats than in *Octodon degus*. The distribution of the total morphine radioactivity in brain, liver and kidney are presented in Table 2. Concentrations of total morphine radioactivity in brain and liver of *Octodon degus* were lower than those of Wistar rats at all the times studied. In the kidney, this level was lower in *Octodon degus* only 30 min after morphine administration, reaching higher values at 60 and 120 min. Concentrations of unchanged morphine and its water soluble metabolites in liver and kidney are presented in Table 3. Liver concentrations of unchanged morphine were lower than its water soluble metabolites at all the times studied in both rodents, but both concentrations were insignificantly lower in *Octodon degus*. However, kidney concentrations of unchanged morphine were lower in *Octodon degus* only 30 min after morphine administration while at 60 and 120 min these concentrations were similar in both rodents. At 30 min, concentrations of water soluble metabolites in the kidney were similar in both rodents but at 60 and 120 min these concentrations were significantly higher in the *Octodon degus* than in the Wistar rats. Interestingly, the ratios of unchanged morphine to water soluble metabolites in the liver did not differ significantly between both rodents at all the times studied. However, in the kidney all of these ratios were significantly lower in the *Octodon degus*.

Table 1. Blood levels of morphine after s.c. administration of [N-methyl-^{14}C]morphine

	Morphine concentration (μg/ml of blood)		
	Wistar rat	*Octodon degus*	*P*
30 min	1.69 ± 0.202	1.62 ± 0.211	>0.6
60 min	1.01 ± 0.080	0.99 ± 0.051	>0.6
120 min	0.39 ± 0.028	0.26 ± 0.037	<0.02

Animals were injected s.c. with morphine (7.5 mg/kg, 10 μCi/kg) and killed after 30, 60 and 120 min by decapitation. Blood was obtained of the injury of neck and unchanged [N-methyl-^{14}C]-morphine extracted and counted as described in "Methods". Each value represents the mean ± SE of 5 animals.

Hepatic microsomal metabolism of morphine

Studies of morphine metabolism *in vitro* were directed to establish whether differences in concentrations of unchanged morphine and its metabolites in the liver and kidney were a result of different metabolic abilities existing between these animals.

Table 2. Distribution of total ^{14}C after s.c. administration of [N-methyl-^{14}C]morphine

	Total Radioactivity (μg/g of wet tissue)		
	Wistar rat	*Octodon degus*	*P*
Brain			
30 min	0.46 ± 0.019	0.29 ± 0.078	<0.05
60 min	0.47 ± 0.019	0.38 ± 0.022	<0.02
120 min	0.48 ± 0.040	0.35 ± 0.053	<0.05
Liver			
30 min	9.07 ± 2.30	4.86 ± 0.30	<0.05
60 min	8.22 ± 0.62	5.08 ± 0.20	<0.04
120 min	5.68 ± 0.22	3.38 ± 0.57	<0.01
Kidney			
30 min	24.20 ± 1.73	15.60 ± 2.55	<0.05
60 min	17.16 ± 3.20	25.02 ± 2.20	<0.05
120 min	6.63 ± 0.34	12.05 ± 2.28	<0.05

Animals were injected s.c. with morphine (7.5 mg/kg, 10 μCi/kg) and killed 30, 60 and 120 min after administration. Aliquots of 20% of tissue homogenates were digested and radioactivity determined in a liquid scintillation counter as described in "Methods".

Each value represents the mean ± SE of 3 animals. Total ^{14}C is expressed as equivalent μg of morphine per gram of wet tissue.

Table 3. Levels of unchanged morphine and its water soluble metabolites after s.c. administration of [N-methyl ^{14}C]morphine

	Unchanged morphine (μg/g of wet tissue)			Water soluble metabolites (μg/g of wet tissue)		
	Wistar rat	*Octodon degus*	*P*	Wistar rat	*Octodon degus*	*P*
Liver						
30 min	1.96 ± 0.369	0.84 ± 0.102	<0.01	6.58 ± 1.260	3.78 ± 0.320	<0.05
60 min	1.78 ± 0.143	1.09 ± 0.163	<0.01	6.07 ± 0.171	3.75 ± 0.242	<0.01
120 min	1.04 ± 0.130	0.48 ± 0.140	<0.01	4.48 ± 0.125	2.74 ± 0.308	<0.02
Kidney						
30 min	16.50 ± 2.620	8.86 ± 0.805	<0.01	6.36 ± 1.840	5.97 ± 1.380	>0.5
60 min	9.95 ± 1.770	8.98 ± 0.980	>0.2	5.83 ± 0.570	13.80 ± 1.970	<0.05
120 min	3.39 ± 3.060	3.25 ± 0.625	>0.2	3.06 ± 0.125	8.05 ± 1.170	<0.05

Animals were injected s.c. with morphine (7.5 mg/kg, 10 μCi/kg) and killed 30, 60 and 120 min after administration. Extraction method was used to separate water soluble metabolites in 20% liver homogenate of unchanged morphine.

Unchanged morphine represents the soluble radioactivity in organic solvent expressed as equivalent μg of morphine/g wet tissue. Water soluble metabolites represents the soluble radioactivity in water expressed as equivalent μg of morphine/g wet tissue. Each value represents the mean ± SE of 3 animals.

Morphine glucuronidation and N-demethylation represent the main metabolic pathway of this compound (Way and Adler, 1961). The responsible enzymes of this reactions are localized mainly in the hepatic microsomal fraction. The hepatic microsomal protein and cytochrome *P*-450 contents were determined in both rodents and the results are shown in Table 4. Liver microsomal protein and cytochrome *P*-450 contents were two times higher in *Octodon degus* than in Wistar rats.

The results of morphine glucuronidation and N-demethylation are presented in Table 5. No differences were observed between both rodents when the V_{max} of morphine glucuronidation were expressed as nmoles of glucuronide formed per min per mg of protein. However, since liver microsomal protein concentration in *Octodon degus* is double that in Wistar rats, as seen in Table 4, morphine glucuronidation expressed per gram of liver in *Octodon degus* appears to be double. Similarly, no differences in morphine N-demethylation were observed between these rodents when V_{max} are expressed as nmoles of

Table 4. Liver protein contents of Wistar rat and *Octodon degus*

	Wistar rat	*Octodon degus*	Ratio Wistar rat/*O. degus*
Liver wt/100 g body wt	4.61 ± 0.54	3.98 ± 0.53	0.86
mg microsomal protein/g wet liver	3.45 ± 0.21	6.24 ± 0.42	1.81
nmol cyt. *P*-450/mg microsomal protein	0.69 ± 0.108	1.45 ± 0.08	2.10

Microsomal fractions were prepared as indicated in "Methods". Protein contents were determined according to Lowry *et al.* (1951). Cytochrome *P*-450 was measured by the method described by Omura and Sato (1964a, b).

Values represents the mean ± SE of 10 experiments.

Table 5. *In vitro* metabolism of morphine in liver microsomes of Wistar rat and *Octodon degus*

	Glucuronidation			N-demethylation		
	Wistar rat	*Octodon degus*	*P*	Wistar rat	*Octodon degus*	*P*
Apparent V_{max} nmol product formed/min/nmol cyt. *P*-450	—	—	—	5.5 ± 0.82	5.5 ± 0.78	<0.01
nmol product formed/min/mg microsomal protein	3.0 ± 0.20	3.0 ± 0.18	<0.01	3.8 ± 0.42	8.0 ± 0.75	<0.01
nmol product formed/min/g wet liver	10.5 ± 0.98	19.0 ± 1.22	<0.01	12.5 ± 1.45	50.0 ± 3.62	<0.01
Apparent K_m (mM)	0.46 ± 0.08	0.46 ± 0.09	>0.7	1.1 ± 0.23	1.1 ± 0.36	>0.6

Microsomes were prepared from livers of Wistar rats and *Octodon degus* as indicated in "Methods". UDP-glucuronyltransferase activity was expressed as nmol of morphine glucuronide, and N-demethylase activity as nmol of formaldehyde formed. Both activities were determined as described in "Methods". All values were calculated from Lineweaver Burk plots. Each value is the mean ± SE of at least four experiments from the pooled livers of four animals in each experiment.

formaldehyde formed per minute per nmol of cytochrome *P*-450. However, considering the higher content of cytochrome *P*-450 per milligram of microsomal protein and the higher content of microsomal protein in *Octodon degus*, the overall N-demethylation is about four times higher in this rodent than in Wistar rats.

DISCUSSION

Previous studies have shown resistance to morphine effects in *Octodon degus* as compared to rat and guinea-pig (Villanueva *et al.*, 1980). The present work suggested that this natural tolerance of *Octodon degus* could be due to a more rapid enzymatic metabolism of morphine in this rodent as compared to Wistar rats.

The distribution pattern of morphine in rats was in general according to values shown in earlier reports (Way and Adler, 1961).

The measurement of total morphine radioactivity in tissues after morphine injections showed a higher concentration in the kidney than in the liver in both rodents, at all time intervals studied. The *Octodon degus* showed a different distribution pattern of morphine than the Wistar rats. In the *Octodon degus* either the amount of unchanged morphine and that of its water soluble metabolites were lower than in rats. The ratios between concentrations of unchanged morphine and its water soluble metabolites in the liver was similar in both rodents, however the analysis of these ratios in the kidney show higher values in the Wistar rats than in the *Octodon degus*. These results suggest an *in vivo* accelerated metabolism of morphine in *Octodon degus*. The lower brain concentration of total ^{14}C and the lower blood concentration of morphine at the time interval of 120 min seen in *Octodon degus* may corroborate this suggestion. However, the lower brain concentrations of total ^{14}C of the *Octodon degus* could also be due to the existence of a difficult uptake of morphine into the brain provoked by a possible less efficient passage through the blood-brain barrier.

Morphine is extensively metabolized by the liver microsomal enzyme system, resulting mainly in the production of a water soluble glucuronide conjugate which is excreted in bile and urine. The second product is an N-demethylated metabolite, the normorphine, which can be later transformed to a glucuronide conjugate or excreted as normorphine. Glucuronide metabolites of morphine have been found to be pharmacologically inactive. However, normorphine presents some pharmacological effects of morphine; the extension and intensity of them depend of the administration route and it biotransformation pathway (Jöhannesson and Milthers 1962; Beckett *et al.*, 1956; Way and Adler, 1961). Apparent V_{max} for morphine glucuronidation and morphine N-demethylation expressed per gram of fresh liver were respectively two and four times higher in *Octodon degus* as compared to those of Wistar rats. These results correspond exactly to the differences observed in the contents of microsomal protein and cytochrome *P*-450 per gram of fresh liver, which were respectively two and four times higher in *Octodon degus*. On the other hand, the apparent K_m of these activities were the same in both rodents. These results appear to indicate that the specificities related to the substrate affinities of UDP-glucuronyltransferase and cytochrome *P*-450 for morphine are similar in both rodents. Similar results as those observed for morphine biotransformation were obtained with other substrates such as *p*-nitrophenol glucuronidation, aminopyrine N-demethylation, *p*-nitroanisol O-demethylation (unpublished results). These results indicate that *Octodon degus* has a higher drug metabolizing ability than Wistar rats. The principal explanation of it may reside in the higher content of the responsible enzymes since the enzymatic activity expressed in nmoles of product formed per milligram of protein did not differ in either rodent. This mechanism may be a factor in the greater tolerance to morphine observed in *Octodon degus* as possibly to other drugs that have not been studied at this moment.

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