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Analgesic Effect of Morphine Glucuronides*

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SHIMOMURA, K., KAMATA, O., UEKI, S., IDA, S., OGURI, K., YOSHIMURA, H. and TSUKAMOTO, H. *Analgesic Effect of Morphine Glucuronides.* Tohoku J. exp. Med., 1971, **105** (1), 45–52 —— Pharmacological properties of morphine-3-glucuronide and morphine-6-glucuronide were studied in mice and compared with those of morphine. 1) The analgesic effect of morphine-6-glucuronide was 3 to 4 times as potent and approximately 2 times as long in duration as that of morphine when injected subcutaneously. Morphine-3-glucuronide, however, showed no analgesic effect even in a dose of 27.6 mg/kg. 2) The analgesic effect of morphine-6-glucuronide was about 45 times as potent as that of morphine, when injected intracerebrally in mice, but morphine-3-glucuronide had no effect. 3) After intraperitoneal injection of morphine-6-glucuronide in rats, only conjugated morphine was detected in the brain by chromatographic examination but not free morphine. 4) Morphine-6-glucuronide was also antagonized by nalorphine though to slightly lesser degree than morphine. 5) Development of tolerance to morphine-6-glucuronide in analgesic effect was almost the same in degree as that to morphine. Cross tolerance between morphine-6-glucuronide and morphine was observed as well. ———— morphine; morphine glucuronides; analgesics; morphine tolerance

It has been well known that the main metabolites of morphine are morphine-3-glucuronide (Fujimoto and Way 1957, 1958), normorphine (Elliott *et al.* 1954, March and Elliott 1954) and codeine (Ellison and Elliott 1964) in various animal species. Quite recently Fujimoto and Haarstad (1969) proved that morphine-3-sulfate was the main metabolite of morphine in the chicken and cat. Almost simultaneously with these authors, Yoshimura *et al.* (1969, a, b) found that morphine was metabolized mainly to morphine-3-glucuronide, and also to morphine-6-glucuronide in a small amount in the rabbit, guinea pig, mouse and human.

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These glucuronides, in general, have been regarded as detoxicated end-products without any pharmacological activity. In fact, morphine-3-glucuronide was reported to have no analgesic action (Woods 1964). Recent studies (Calvin *et al.* 1963, Calvin and Lieberman 1964, Lebeau *et al.* 1964, Robert *et al.* 1964), however, strongly suggested that the ethereal sulfates or glucuronides of certain steroid hormones were not only the urinary metabolites, but also were possibly utilized as biosynthetic precursors of the other steroid hormones. In addition, Casparis (1950) found an interesting fact that morphine-6-glucoside showed a higher analgesic activity than morphine itself in mice.

From these findings, it seemed worthwhile to examine the pharmacological activity of morphine-6-glucuronide. The present paper deals with the pharmacological property, especially the analgesic effect of morphine-6-glucuronide as well as of morphine-3-glucuronide.

Materials and Methods

Animals: Male *dd*-strain mice of 18–23 g in body weight were used in the analgesic experiments. For the biochemical study, Donryu-strain rats weighing 140–150 g were used.

Measurement of analgesic effect: The analgesic effect in mice was determined by Haffner's (Haffner 1929) and the hot plate methods (Woolfe and Macdonald 1944). In Haffner's method, the tail of mice was pinched by a clamp with fixed pressure of 500 g. When the mouse turned the head backward and tried to bite the clamp within 5 seconds after pinching, the pain response was regarded as positive. In the hot plate method, the temperature of the plate was maintained at 55°C. A criterion of the pain response was either licking the hind paw or jumping on the hot plate, and the latency of the response was measured twice at an interval of 30 minutes prior to the drug injection. The mice which showed the pain response within 13 seconds were used in the experiment. When the latency of pain response was increased to more than twice the pre-drug value, analgesia was regarded as positive. The mice were never kept on the hot plate for more than 30 seconds even when no pain response occurred. The drugs were not only injected subcutaneously but also intracerebrally to examine their analgesic effect. Intracerebral injection was performed through the needle impaled 3 mm deep into the parietal cortex. Total volume of the drug solution was 0.025 ml. No effect was observed by intracerebral injection of this volume of physiological saline. The data were statistically analyzed by Litchfield-Wilcoxon's method (Litchfield and Wilcoxon 1949).

Acute toxicity: The lethal effects of morphine-6-glucuronide (M-6-G) and morphine, and the LD50s were determined by the up and down method (Brownlee *et al.* 1953).

Detection of morphine and its glucuronides in the rat brain: Presence of free and conjugated morphine in the brain was qualitatively examined 45 minutes after an intraperitoneal injection of morphine-6-glucuronide in dose of 30 mg/kg, using 19 male Donryu-strain rats. The brain was extirpated after perfusion with physiological saline through the carotid artery. The homogenate of the total brain (wet weight was 18.5 g) in 74 ml of 12.5% HCl solution was centrifuged at 15,000 g for 5 minutes. The precipitate was extracted twice more with 10% HCl solution similarly as above. The combined supernatants were adjusted to pH 9.0 with $NaHCO_3$ and extracted 3 times with a half volume of $CHCl_3$-isoPrOH (3:1).

The residue obtained after evaporation of the organic solvents *in vacuo*, was dissolved

in diluted HCl solution, and non-basic impurities were extracted out with $CHCl_3$. The HCl solution was then made alkaline with $NaHCO_3$ and evaporated to dryness *in vacuo*. The resulting residue was dissolved in a small volume of MeOH and it was used as the sample for determination of free morphine (sample I).

The aqueous alkaline layer, remained after extraction of the supernatant with $CHCl_3$-isoPrOH, was made 5% acid concentration by addition of an appropriate volume of conc. HCl and hydrolyzed at 122°C for 30 minutes in an autoclave. Under this hydrolytic condition, virtually complete liberation of morphine from morphine-6-glucuronide was confirmed. The liberated morphine in the hydrolysate was extracted at pH 9.0 with $CHCl_3$-IsoPrOH (3:1) similarly as above. This extract was spotted in a line on a plate (20×20 cm) of silica gel G, Merck, 0.25 mm thick, activated at 105°C for 30 minutes and then developed with the solvent system of $CH_2Cl \cdot CH_2Cl$-MeOH (2:1). After visualizing one side of the chromatogram with potassium platinum iodide reagent, the band corresponding to morphine (Rf, 0.40) was scraped off of the remaining side into a centrifuge tube, and morphine was extracted 3 times with 10 ml of MeOH. The MeOH extract was concentrated to a small volume and used as the sample for examination of morphine-6-glucuronide (sample II).

Presence of morphine in both samples I and II was examined by thin-layer chromatography using the solvent system of CH_2Cl CH_2Cl-MeOH (2:1) and EtOH-dioxane-benzene-conc. NH_4OH (5:40:50:5. upper layer). Morphine could be detected in a microgram quantity by either fluorescence or coloration with potassium platinum iodide reagent (Yoshimura *et al.* 1966). Rf values of morphine in the former and latter solvent systems were 0.40 and 0.25, respectively.

Drugs: Morphine-6-glucuronide (M-6-G) and morphine-3-glucuronide (M-3-G), used in this experiment, were synthesized chemically (Yoshimura *et al.* 1968, Oguri *et al.* 1970). Morphine hydrochloride, nalorphine hydrochloride were also used. All the drugs were dissolved in physiological saline solution.

Results

Analgesic effect

Analgesic effects of M-6-G and morphine, measured by the hot plate method, were illustrated in Fig. 1. The effect of M-6-G was more potent than that of morphine. The peak effect of both drugs was attained about an hour after subcutaneous injection but the effect of M-6-G was much longer in duration than that of morphine. Similar results were obtained by the measurement using Haffner's method. The ED50s of M-6-G and morphine were calculated from the analgesic potency measured at the peak time by the hot plate and Haffner's methods. The results were shown in Table 1. It is clear, from this result, that M-6-G is approximately 3.7 times as potent as morphine. M-3-G showed no analgesic effect even in a subcutaneous dose of 27.6 mg/kg.

The analgesic effects of M-6-G and morphine were also determined by Haffner's method 15 minutes after intracerebral injection. The dose-effect relationship, illustrated in Fig. 2, showed that M-6-G exerted an extremely potent analgesic effect when injected intracerebrally, as compared with morphine; in fact, M-6-G was approximately 45 times as potent as morphine. No effect was observed with M-3-G upon intracerebral injection even in a dose of 10 μg/body.

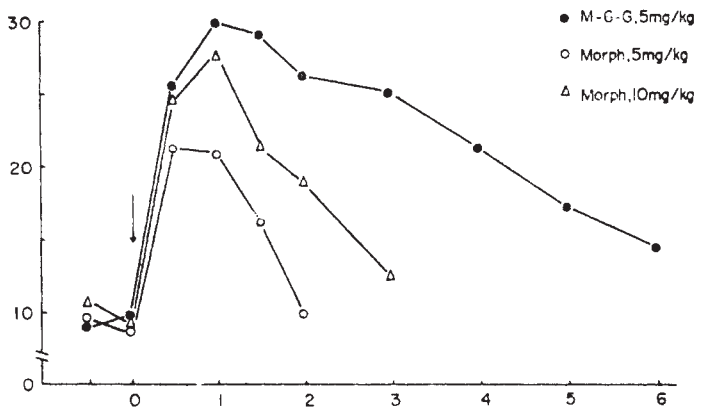

Fig. 1. The analgesic effects of M-6-G and morphine (hot plate method). The drugs were injected subcutaneously in mice.
Abscissa: hours. Ordinate: seconds.

TABLE 1. *ED50 and LD50 of M-6-G and morphine*

Drug	ED50 (mg/kg)		LD50 (mg/kg)
	Hot plate	Haffner's	
Morphine-HCl	4.5 (3.2~6.3)	5.9 (3.9~8.9)	500
Morphine-6-glucuronide	1.2 (0.7~2.0)	1.5 (0.8~2.7)	432

( ): 95% fiducial limits.

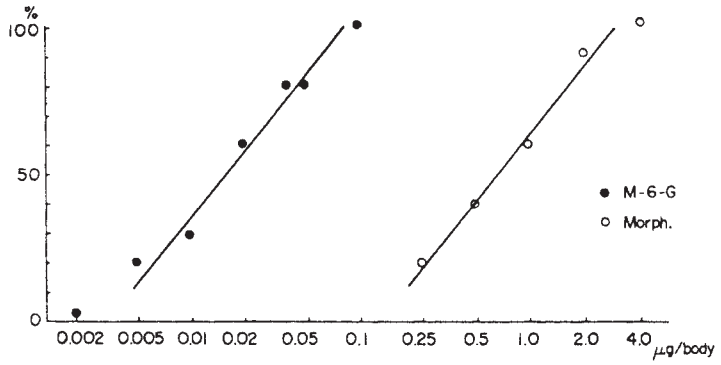

Fig. 2. The analgesic effect of M-6-G and morphine upon intracerebral injection (dose-response curve). The analgesic effect was determined by Haffner's method 15 minutes after the drugs were injected intracerebrally in mice. The ED50s of M-6-G and morphine were 0.016 μg/body (0.009 –0.027 μg/body) and 0.70 μg/body (0.44–1.12 μg/body) respectively.

Acute toxicity

The lethal effects of M-6-G and morphine were determined 24 hours after subcutaneous injection, and the LD50s were calculated. The LD50 of M-6-G was only slightly smaller than that of morphine (Table 1).

Antagonism by Nalorphine

The antagonistic effect of nalorphine against the analgesia induced by M-6-

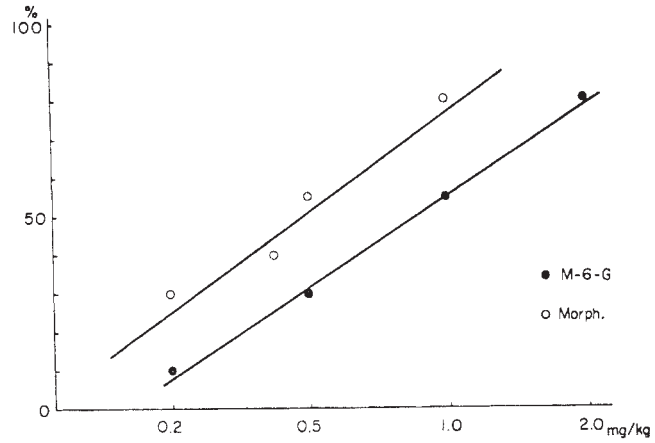

Fig. 3. Antagonism by nalorphine against the analgesic effect of M-6-G and morphine. Nalorphine was injected subcutaneously 30 minutes after subcutaneous injection of M-6-G (3.5 mg/kg) and morphine (10 mg/kg).

G and morphine was examined by the hot plate method in mice. Various doses of nalorphine were injected subcutaneously 30 minutes after subcutaneous injection of M-6-G (3.5 mg/kg) and morphine (10 mg/kg) (10 mg/kg of morphine was almost equivalent in analgesic potency to 3.5 mg/kg of M-6-G). The analgesia was measured 30 minutes after the administration of nalorphine and the dose of nalorphine sufficient to cause 50% inhibition of the analgesic effect of either M-6-G or morphine was obtained. The ED50 of nalorphine was 0.80 mg/kg (0.51–1.26 mg/kg) for M-6-G and 0.48 mg/kg (0.29–0.81 mg/kg) for morphine. M-6-G was antagonized by nalorphine to lesser degree as compared with morphine, though this difference in the ED50s was not significant statistically.

Tolerance development

Tolerance development in the analgesic effect of M-6-G was examined and compared with that of morphine. M-6-G (3.5 mg/kg) or morphine (10 mg/kg) were injected subcutaneously once a day in each group of 10 mice and the analgesic effect was measured every other day by the hot plate and Haffner's methods, 60 minutes after the drug administration. Tolerance to both 3.5 mg/kg of M-6-G and 10 mg/kg of morphine developed rapidly in a period of 7 days, thus the dose of M-6-G was increased to 7.0 mg/kg and that of morphine to 20 mg/kg, but tolerance developed to these doses in a subsequent period of 10 days as shown in Fig. 4. Cross tolerance was also observed between morphine and M-6-G, when tested on the 19th day.

Detection of M-6-G in the brain.

Forty-five minutes after intraperitoneal injection of M-6-G in a dose of 30 mg/kg in Donryu strain rats, only conjugated morphine was detected in the brain. This result suggested that M-6-G penetrated into the brain with unchanged form, and was not changed to free morphine by hydrolysis.

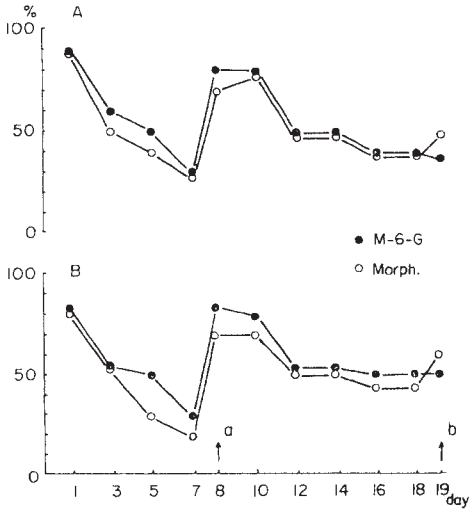

Fig. 4. Changes in the analgesic effects of M-6-G and morphine upon chronic administration. M-6-G (3.5 mg/kg) or morphine (10 mg/kg) were injected once a day subcutaneously.
A: Hot plate method, B: Haffner's method.
a: The doses of M-6-G and morphine were increased to 7.0 mg/kg and 20 mg/kg respectively.
b: Morphine group was exchanged to M-6-G (7.0 mg/kg) and M-6-G group to morphine (20 mg/kg).

Discussion

It has been a general concept that glucuronides are easily excreted in urine with their high water-solubility and have no pharmacological activity. The fact that M-3-G had not analgesic effect by either subcutaneous or intracerebral injection, as shown in the present investigation, seemed to agree well with this concept. It was, however, very interesting that the effect of M-6-G was much more potent and was longer in duration than that of morphine, when injected subcutaneously. This fact could not be explained by this concept.

In our present investigation, free morphine was never detected, but only bound morphine was obtained in the brain after the systemic administration of M-6-G. This result strongly suggested that M-6-G could penetrate the blood-brain barrier and affect the side of action of morphine. The penetration of M-6-G into the brain structure seemed very poor as compared with that of morphine, because the potency of M-6-G was about 45 times upon intracerebral injection, whereas about 3.7 times that of morphine upon systemic administration. M-6-G seemed to act much more strongly after penetration into the brain and to be removed more slowly from the brain as compared with morphine, because the effect of M-6-G was much longer in duration than that of morphine.

Tolerance development to M-6-G was found to be almost the same as that to morphine after repeated subcuteneous injection and cross tolerance to both the drugs was also observed.

When calculated from the ratio of ED50 to LD50, the saiety margin of M-6-G

was about 3 times that of morphine. Therefore, M-6-G seemed to be superior to morphine in this respect as an analgesic drug.

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