Although the mechanism of this cyclization is not yet clear, the formation of theophyllines presumably involves the oxidative process by the initial formation of the sulfinyl chloride intermediates (IIIa—f) and subsequent cyclization by the loss of hydrogen chloride and sulfur monoxide. The elimination of hydrogen chloride and sulfur monoxide has also been postulated in certain sulfinyl chlorides[8a-c] (Chart 1).

Experimental[9]

6-Amino-5-benzylideneamino-1,3-dimethyluracils (IIa—f) (Table I)——A mixture of 5,6-diamino-1,3-dimethyluracil (I)[5] (0.51 g, 0.003 mole) and an equimolar amount of respective aldehydes in EtOH (20 ml) was refluxed for 1 hr. After cooling, the precipitated solid was filtered and recrystallized from EtOH to give the corresponding pure product (IIa—f).

8-Substituted Theophyllines (IVa—f) (Table II)——A mixture of 6-amino-5-benzylideneamino-1,3-dimethyluracil (0.003 mole) and $SOCl_2$ (5 ml) was refluxed for 5 min. The reaction mixture was evaporated *in vacuo*, and the residue was triturated with aqueous ammonia to give a solid. Recrystallization from dimethylformamide (DMF) gave the corresponding pure product (IVa—f).

8) *a*) A.J. Krubsack and T. Higa, *Tetrahedron Letters*, **1968**, 5149; *b*) H.M. Relles, *J. Org. Chem.*, 23, 3630 (1972); *c*) A.J. Krubsack and T. Higa, *Tetrahedron Letters*, **1973**, 4515.

9) Melting points were taken on a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Japan Spectroscopic Co., Ltd. spectrophotometer, Model IR-E from samples mulled in Nujol.

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Effect of Morphine and Its Conjugates on the Isolated Ileal Preparation of Guinea-pig

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The present study was undertaken to reconfirm the previous conclusion that morphine-6-conjugates cause potent analgesia by themselves in mice. For this purpose, inhibitory effect of morphine and its three pairs of 3- and 6-conjugates (glucuronides, ethereal sulfates and phosphates) to nicotine-induced contraction of the isolated guinea-pig ileum was examined. The potency of these conjugates was found to decrease in the following order, morphine-6-sulfate> morphine-6-glucuronide> morphine, morphine-3- and 6-phosphate> morphine-3-glucuronide, morphine-3-sulfate, indicating fairly good accordance with order in their analgesic effects in mice. The present experiment again provided strong suggestion that the above biological effect was attributable to the conjugates themselves.

Keywords——morphine; morphine glucuronides; morphine sulfate esters; morphine phosphate esters; ileum contraction; isolated guinea-pig ileum

Metabolism of morphine has been extensively studied by many workers to elucidate mechanism of the action and development of the tolerance and dependence. Yoshimura, *et al.*[2] showed for the first time that several mammalian species including man excreted morphine-6-

1) Location: *a*) *1-14 Bunkyo-machi, Nagasaki*; *b*),*c*) *3-1-1 Maidashi, Higashi-ku, Fukuoka*; *d*) The author to whom reprint requests should be sent.

2) H. Yoshimura, K. Oguri, and H. Tsukamoto, *Biochem. Pharmacol.*, **18**, 279 (1969); K. Oguri, S. Îda, H. Yoshimura, and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 2414 (1970).

glucuronide (M-6-G) as a minor urinary metabolite of morphine, along with the major metabolite, morphine-3-glucuronide (M-3-G). Fujimoto, *et al.*[3] and Yeh, *et al.*[4] also reported that morphine-3-sulfate (M-3-S) was mainly excreted in the excreta of cat and chicken treated with morphine.

In general, these conjugates have been believed to be the detoxicated metabolites. For example, M-3-G, the major metabolite of morphine, was previously reported by Woods[5] to have no pharmacological activity. Although Sasajima[6] reported that intracerebral injection of M-3-G induced analgesia in mice, Schultz and Goldstein[7] suggested that this analgesia was caused by the free base resulting from hydrolysis of M-3-G *in vivo*, but not by the conjugate itself. On the other hand, Shimomura, *et al.*[8] and Mori, *et al.*[9] demonstrated recently that analgesic activity of M-6-G and morphine-6-sulfate (M-6-S) was much higher in potency and longer in duration than that of morphine, according to the Haffner's and hot plate method, but such strong analgesia was not induced by M-3-G and M-3-S. In the subsequent study, Mori, *et al.*[10] showed that either morphine-3-phosphate (M-3-P) or the 6-isomer (M-6-P) revealed the same level of analgesic potency as morphine. It was evidenced that analgesic activity exerted after injection of M-6-G is not mediated by morphine which might be liberated from M-6-G, but by M-6-G itself.[11] The same idea could be extended to M-6-S. In the case of M-3-P and M-6-P, however, it was presumed that the analgesia might be attributable to liberated morphine, because the analgesic potency of both conjugates was just the same as that of morphine.

The present study was undertaken to examine firstly whether M-6-G and M-6-S themselves possess any other pharmacological activity than analgesic activity, and secondly whether the morphine phosphate esters can exert the pharmacological activity by themselves or not. For this purpose the inhibitory effect of morphine and its conjugates on nicotine-induced contraction of the isolated guinea-pig ileum[12] was investigated, since this *in vitro* system was considered to afford more direct response to above conjugates than analgesic examination methods using intact animals.

Materials and Methods

Drugs——Morphine hydrochloride and nicotine (free base) were obtained from commercial sources. M-6-G, M-3-G[13], M-6-S, M-3-S,[9] M-6-P and M-3-P[10] were prepared by the methods reported previously. All the drugs were dissolved in distilled water and the concentrations expressed as final molar concentrations.

Measurement of Contraction——A segment of ileum, 1.5—2.0 cm long which was taken from the ileum of male guinea-pig weighing 350 to 500 g, was suspended in an organ bath containing 25 ml of Tyrode's solution bubbled with air. The temperature of this solution was maintained at 35°. The mechanical response to drugs were measured isometrically by using a straingage (Kyowa, Type KFC-5-Cl-11) and recorded on a potentiometric pen recorder (Shimadzu, model R-11). The initial tension of ileum was adjusted to 0.2—0.3 g. When the size of contraction was plotted against the concentration of nicotine with negative logarithmic scale, the ileum showed a linear response over the range from 1×10^{-6} to 5×10^{-5} M.

For evaluating the inhibitory effect of morphine and its conjugates on this nicotine-induced contraction, the tissue was stimulated with 2.5×10^{-6} M nicotine, which gave 40% of the maximum size of contraction.

3) J.M. Fujimoto and V.B. Haarstad, *J. Pharmacol. Exptl. Therap.*, **165**, 45 (1969).
4) S.Y. Yeh, H.I. Chernov, and L.A. Woods, *J. Pharm. Sci.*, **60**, 469 (1971).
5) L.A. Woods, *J. Pharmacol. Exptl. Therap.*, **112**, 158 (1954).
6) M. Sasajima, *Keio Igaku*, **47**, 421 (1970).
7) P. Schultz and A. Goldstein, *J. Pharmacol. Exptl. Therap.*, **183**, 404 (1972).
8) K. Shimomura, O. Kamata, S. Ueki, S. Îda, K. Oguri, H. Yoshimura and H. Tsukamoto, *Tohoku J. Exptl. Med.*, **105**, 45 (1971).
9) M. Mori, K. Oguri, H. Yoshimura, K. Shimomura, O. Kamata, and S. Ueki, *Life Sci.*, **11**, Part I, 525 (1972).
10) M. Mori, K. Oguri, H. Yoshimura, and O. Kamata, *Yakugaku Zasshi*, **93**, 1302 (1973).
11) H. Yoshimura, S. Îda, K. Oguri, and H. Tsukamoto, *Biochem. Pharmacol.*, **22**, 1423 (1973).
12) H.W. Kosterlitz and J.A. Robinson, *Brit. J. Pharmacol.*, **13**, 296 (1958); G.P. Lewis, *ibid.*, **15**, 425 (1960).
13) H. Yoshimura, K. Oguri, and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2114 (1968); K. Oguri, H. Yoshimura and H. Tsukamoto, *ibid.*, **18**, 209 (1970).

This antagonistic potency was expressed as a percent inhibition (I) and calculated with following formula;

$$I = \frac{A-B}{A} \times 100$$

where A is a peak height from zero position of contraction induced by nicotine alone, before or after testing the potency of morphine or its conjugates (both values must be same); B is a peak height from zero position of contraction induced by nicotine in the presence of morphine or its conjugates (see Fig. 1). As shown in this figure, B was measured from the initial position before the addition of morphine, but not after the addition of morphine, because the former was found experimentally to be more proportional to the dose than the latter. The antagonistic potencies at three different concentrations of morphine or its conjugates were thus determined and plotted semilogarithmically.

Results and Discussion

Besides the action on the central nervous system, morphine is known to depress impulse transmission at certain junctions of autonomic nervous system. For instance, nicotine-induced contraction of the isolated guinea-pig ileum is antagonized by morphine.[12] Utilizing this phenomenon the antagonistic effects of synthesized three pairs of morphine conjugates (3- and 6-isomers of glucuronides, ethereal sulfates and phosphates) were examined as described in the Methods. In Fig. 1 the effect of morphine is illustrated as a typical example.

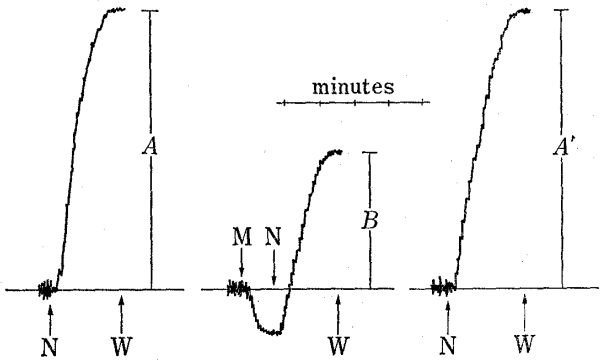

Fig. 1. Illustrative Figure for Measurement of Inhibitory Effect of Morphine (1×10^{-7}M) on the Contraction of Isolated Guinea-pig Ileum induced by Nicotine (2.5×10^{-6}M)

N: addition of nicotine; W: washing with Tyrode's solution; M: addition of morphine; A and A': size of nicotine-induced contraction before and after testing the effect of morphine, respectively; B: size of nicotine-induced contraction in the presence of morphine
See Methods for experimental conditions.

As can be seen in this figure, morphine and its conjugates exhibited, together with antagonistic effect to nicotine, a little decreasing effect on the tonus of ileum, which was possibly produced by the inhibition of the spontaneous electrical activity of nervous system in guinea-pig ileum according to Dingledine and Goldstein.[14]

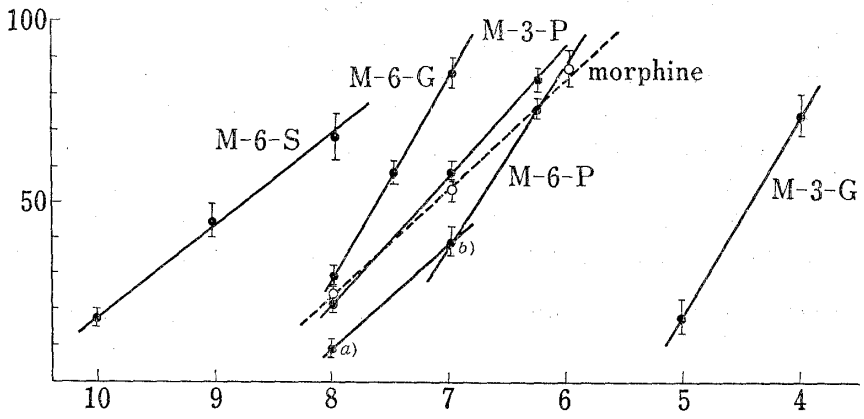

Fig. 2. Inhibitory Effect of Morphine and Its Conjugates on the Contraction of Guinea-pig Ileum induced by Nicotine

abscissa: molar concentration of morphine and its conjugates with a negative logarithmic scale, ordinate: percent inhibition to the contraction induced by 2.5×10^{-6}M nicotine. The standard errors of the means of more than 10 experiments are indicated by the vertical lines.
a) significantly different from morphine, $p < 0.05$
b) significantly different from M-3-P, $p < 0.05$

14) R. Dingledine and A. Goldstein, *Life-Sci.*, **17**, 57 (1975).

Fig. 2 shows the plots of dose dependent inhibitory effects of above morphine derivatives to nicotine-induced contractions. All of these drugs produced linear increases in inhibitory effects in parallel with the concentrations of drugs except for M-6-P, in which the inhibitory curve is bent at the concentration of 10^{-7} M.

Among these conjugates, M-6-S showed the exceedingly high antagonistic potency to nicotine-induced contraction. The potency of the other drugs was followed in the order, M-6-G>morphine=M-3-P≧M-6-P>M-3-G. M-3-S caused tachyphylaxis so rapidly that the precise potency could not be evaluated. It seems, however, that M-3-S possesses almost the same level of antagonistic effect as M-3-G if the tachyphylaxis is ignored. Schultz and Goldstein[7] demonstrated recently that 10^{-7} M levels of M-3-G and levorphanol-3-glucuronide did not influence the tension of the electrically stimulated guinea-pig intestinal preparation.[7] The present study also indicated that M-3-G did not exert the inhibitory effect in this concentration (10^{-7} M) and hundred times higher level (10^{-5} M) of M-3-G was needed to obtain the same potency as morphine.

Since the analgesic potency in mice decreases in the order, M-6-G, M-6-S>morphine, M-3-P, M-6-P>M-3-G, M-3-S, as already reported[8-10], both effects of morphine derivatives on guinea-pig ileum *in vitro* and on mice *in vivo* are in fairly good accordance. However, with regard to M-6-S and M-6-G, the situation is somewhat complicated by the fact that they reveal almost equipotent analgesic effect by subcutaneous or intracerebral injection, but M-6-S is much more potent than M-6-G in the ileal preparation. Considering the facts that M-3-G may not be hydrolyzed to morphine in appreciable amount, if any, during the experiment, because potency of M-3-G is only about one hundredth compared with that of morphine in guinea-pig ileum and that both M-3-G and M-6-G have almost same K_m value to β-glucuronidase,[15] the lower activity of M-6-G than M-6-S is not due to partial hydrolysis of M-6-G to morphine. Thus, it was confirmed that the potency of M-6-S and M-6-G in guinea-pig ileum is higher than that of morphine as in the analgesic potency, however, it is also suggested that for these two conjugates, the mode of action is not completely the same in the central and peripheral nervous systems.

One more question to be answered is whether the biological activity is exerted by M-3-P and M-6-P themselves or by morphine liberated from these conjugates by hydrolysis with phosphatases. Although it is not conclusive, the former idea is more plausible than the latter, because the repressive effect of above phosphate esters on the initial tonus of ileum can be observed immediately (without time lag) after the addition. Antagonistic effect to nicotine was also examined at a very short time (45 sec) after the addition of the phosphates. Furthermore, significant differences were found between the effects of morphine and M-6-P at the concentration of 1×10^{-8} M, and of M-3-P and M-6-P at the concentration of 1×10^{-7} M, respectively. These facts preferably support the assumption that the effects of M-3-P and M-6-P were mainly exerted by themselves. However, the reason why only M-3-P exhibits the same level of effect as that of morphine among morphine-3-conjugates is remained to be further studied.

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15) S. Îda, K. Oguri, and H. Yoshimura, *Yakugaku Zasshi*, **95**, 564 (1975).