Notes

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Involvement of 5-Hydroxytryptamine in Tolerance Development in the Morphine-Induced Straub Tail Reaction[1]

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The tolerance development in Straub tail reaction [STR] on successive treatments with morphine was studied. Morphine at 20 mg/kg, four times a day, produced tolerance in STR more rapidly than 10 mg/kg, four times a day. Significant tolerance in STR developed when morphine was injected daily into the cerebral ventricle in mice. STR in tolerant mice was markedly reduced by treatment with L-5-hydroxytryptophan compared to that in non-tolerant mice. The cortical 5-hydroxytryptamine [5-HT] content in tolerant mice was significantly decreased compared to that in non-tolerant mice. These results suggest that cortical 5-HT may play an important role in tolerance development in STR.

Keywords——morphine; Straub tail reaction; tolerance; 5-hydroxytryptamine; 5-hydroxyindoleacetic acid

It has been reported that the morphine-induced Straub tail reaction [STR][3] is due to stimulation of the spinal cord.[4] Gaddum[5] suggested further that this phenomenon was due to a spasm of the vesical and anal sphincters resulting from spinal stimulation. Kameyama and Ukai[6] proposed that STR may be depressed positively by centrally acting muscle relaxants. In this connection, STR was investigated in relation to the neurotransmitters in the central nervous system [CNS] in mice.[7] It was suggested recently that STR may be elicited by a decrease in tryptaminergic activity or by an increase in catecholaminergic activity in the spinal cord.[7a,c] It is known, on the other hand, that successive administrations of morphine induce tolerance to analgesia[8] and locomotor activity.[9] However, the tolerance development in STR has not been reported in detail. The present study was conducted in an attempt to clarify the underlying mechanism of tolerance development in STR in mice with reference to the tryptaminergic neuronal pathway.

Materials and Methods

Experiments were conducted with ddY mice weighing between 18 and 25 g. Mice were divided into groups of fifteen. Food and water were given freely. The following drugs were used: morphine hydrochloride (Shionogi and Co.) and L-5-hydroxytryptophan [L-5-HTP] (Kyowa Hakko Kogyo Co.). The schedule for

1) A preliminary report of this work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1977.
2) Location: *Tenpaku-ku, Nagoya 468, Japan.*
3) W. Straub, *Dtsch. Med. Wochenschr.*, **37**, 1462 (1911).
4) W.J.R. Heinekamp, *J. Pharmacol. Exp. Ther.*, **20**, 107 (1923).
5) J.H. Gaddum, "Pharmacology," 5td ed., Oxford University Press, London, 1959.
6) T. Kameyama and M. Ukai, *Chem. Pharm. Bull.* (Tokyo), **27**, 1063 (1979).
7) *a*) T. Kameyama, M. Ukai, and T. Nabeshima, *Jpn. J. Pharmacol.*, **28**, 249 (1978); *b*) T. Kameyama, M. Ukai, and T. Nabeshima, *Chem. Pharm. Bull.* (Tokyo), **26**, 770 (1978); *c*) T. Kameyama, T. Nabeshima, M. Ukai, and K. Yamaguchi, *ibid.*, **26**, 2615 (1978); *d*) T. Kameyama, M. Ukai, and T. Nabeshima, *ibid.*, **26**, 3265 (1978).
8) E.L. Way, H.H. Loh, and F. Shen, *Science*, **162**, 1290 (1968).
9) T. Kameyama and H. Hori, Abstract of the 4th Symposium on Pharmacological Activity and Mechanism, Hiroshima, 1975, p. 89.

morphine administration (4/day) is shown in Fig. 1. Morphine was also administered twice a day in some cases, *i.e.*, at 9: 30 and 18: 30. Daily intracerebral injection of morphine was carried out once at 15: 30. The intensity of STR was measured according to the method of Juul[10] as follows: 0=0°, 0.5=1—44°, 1.0=45°, 1.5=46—89°, 2.0=90°, 2.5=91—179°, 3.0=180° above the horizontal plane. For biochemical investigation, mice were decapitated 30 min after the final administration of morphine. The brain was divided into 3 regions consisting of the cerebral cortex, mesencephalon plus diencephalon, and pons plus medulla oblongata, according to the method of Glowinski and Iversen.[11] The spinal cord was dissected into two regions, *i.e.*, the thoracic and lumbosacral cords. 5-Hydroxytryptamine [5-HT] and 5-hydroxyindoleacetic acid [5-HIAA] contents were determined by the method of Curzon and Green.[12] Statistical significance was determined by means of Student's *t*-test.

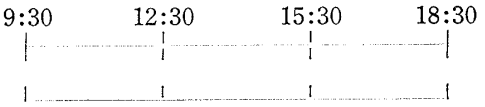

Fig. 1. Experimental Procedure for Successive Morphine Administrations in One Day

See the text.

Results

Effect of Successive Administrations of Morphine on STR

As shown in Fig. 2, there was no significant difference in the tolerance development in STR on injection of morphine, 10 mg/kg, either twice a day or four times a day. On injection four times a day, morphine at 20 mg/kg produced tolerance in STR more rapidly than morphine at 10 mg/kg. Evidently, mice developed tolerance to morphine (20 mg/kg, *s.c.*, 4/day) quite effectively.

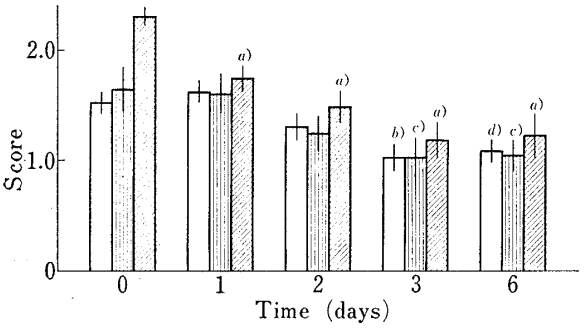

Fig. 2. Effect of Daily Successive Administrations of Morphine on STR

The intensity of STR was determined 30 min after the first injection of morphine each day. Each vertical column represents the mean of fifteen mice. Vertical bars represent the standard errors of the means. ☐: morphine 10 mg/kg, *s.c.* 2/day. ▥: morphine 10 mg/kg, *s.c.* 4/day. ▨: morphine 20 mg/kg, *s.c.* 4/day. The Straub score after the priming dose of morphine is shown as *a*), *b*), $p<0.01$; *c*), *d*), $p<0.05$ compared to the effect of the priming dose of morphine.

Effect of Daily Intracerebral Injection of Morphine on STR

As illustrated in Fig. 3, involvement of the CNS in the tolerance development was demonstrated. Morphine at 40 μg/mouse was used for successive intracerebral injections, since morphine at 40 μg/mouse was shown to elicit the maximum effect in an acute experiment.[6] The intensity of STR was determined at 15, and 30 min after the daily intracerebral injection of morphine. STR was significantly inhibited at 15 min after the third injection of morphine. STR scarcely developed after the fifth injection of morphine.

Effect of L-5-HTP on STR in Non-Tolerant and Tolerant Mice

The effect of L-5-HTP on STR in non-tolerant mice was evident (Fig. 4), as previously described elsewhere.[7a] STR in tolerant mice was significantly attenuated by treatment with L-5-HTP compared to that in non-tolerant mice. The inhibitory rates of L-5-HTP on STR in non-tolerant and tolerant mice were 26 and 76%, respectively.

Effect of Successive Subcutaneous Administration of Morphine on the Levels of 5-HT and 5-HIAA in Various Regions of the Brain and Spinal Cord

Figure 5 shows the intensity of STR immediately before decapitation. The cortical 5-HT content in tolerant mice was decreased, compared to that in non-tolerant mice. There was

10) A. Juul, *Arch. Int. Pharmacodyn. Ther.*, **62**, 69 (1939).
11) J. Glowinski and L.L. Iversen, *J. Neurochem.*, **13**, 655 (1966).
12) G. Curzon and A.R. Green, *Brit. J. Pharmacol. Chemother.*, **39**, 653 (1970).

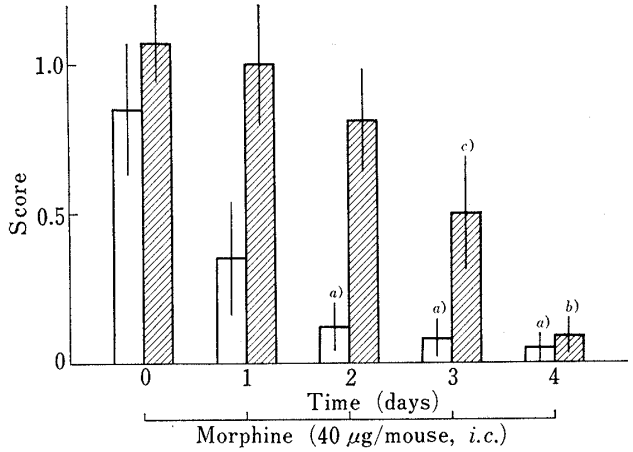

Fig. 3. Effect of Daily Intracerebral Injection of Morphine on STR

Morphine (40 μg/mouse) was injected once a day. Each vertical column represents the mean of fifteen mice. Vertical bars represent the standard errors of the means. □: 15 min post-morphine. ▨: 30 min post-morphine. *a*), *b*), $p<0.01$; *c*), $p<0.05$ compared to the effect of the priming dose of morphine.

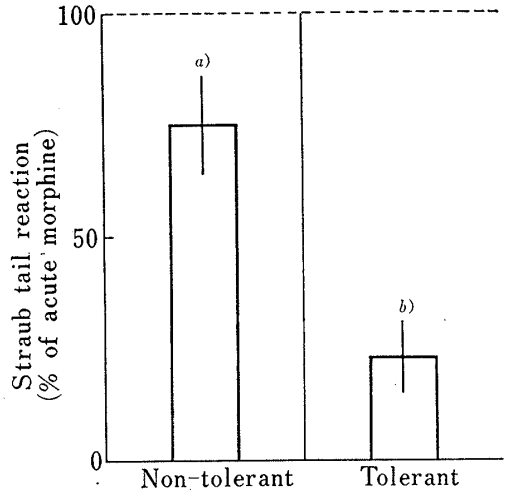

Fig. 4. Effect of L-5-Hydroxytryptophan [L-5-HTP] on STR in Non-Tolerant and Tolerant Mice

L-5-HTP (100 mg/kg, *i.p.*) was given 1 hr before the administration of morphine. Each vertical column represents the mean of fifteen mice. Vertical bars represent the standard errors of the means. *a*), $p<0.05$; *b*), $p<0.01$ compared to the effect of acute morphine.

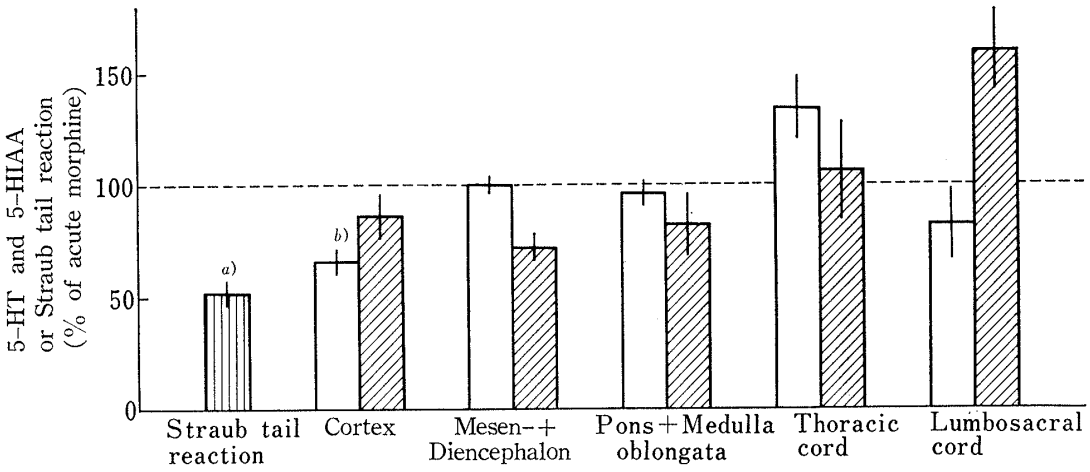

Fig. 5. Effect of Successive Subcutaneous Administrations of Morphine on the Levels of 5-Hydroxytryptamine [5-HT] and 5-Hydroxyindoleacetic Acid [5-HIAA] in Various Regions of the Brain and Spinal Cord

Tolerance in STR was developed by treatment with morphine (10 mg/kg, 4/day) for 5 days and with subsequent morphine (20 mg/kg, 4/day) for 9 days. Mice were decapitated on the 15 th day after priming. Each vertical column represents the mean of five samples. Vertical bars represent the standard errors of the means. ▥: STR. □: 5-HT. ▨: 5-HIAA. *a*), *b*), $p<0.01$ compared to the effect of acute morphine.

no significant change in 5-HT and 5-HIAA contents in regions of the brain and spinal cord other than the cerebral cortex.

Discussion

It has been reported that drug concentration in animals must be maintained at a certain level in order to develop tolerance.[13] Morphine at 20 mg/kg, four times a day, developed tolerance in STR more rapidly than morphine at 10 mg/kg, four times a day, although there

13) H. Kaneto, K. Shimomura, C. Kamei, and H. Nakanishi, *Nippon Yakurigaku Zasshi*, **66**, 487 (1970).

was no significant difference in tolerance development in STR on administration of morphine (10 mg/kg), either twice a day or four times a day. Hence, it is possible that the greater the tissue concentration of morphine given to the mice, the faster the tolerance development in STR. Furthermore, tolerance in STR was observed clearly on daily injection of morphine into the cerebral ventricle in mice. This finding supports the view that the tolerance in STR may be developed in the CNS in mice.

It has previously been reported that an increase in 5-HT activity in the CNS prevents STR.[7a] It has also been suggested, on the contrary, that 5-HT may be important for tolerance development induced by morphine in contrast to other proposed neurotransmitters, *i.e.*, acetylcholine, dopamine and norepinephrine.[14] The cortical 5-HT content in tolerant mice was decreased, compared to that in non-tolerant mice, without any change in cortical 5-HIAA content. There was no significant change in 5-HT or 5-HIAA content in the mesencephalon plus diencephalon, pons plus medulla oblongata, or in the thoracic or lumbosacral cord. Therefore, the marked inhibition of STR by L-5-HTP in tolerant mice compared to that in non-tolerant mice might be attributed to post-synaptic hyperexcitability[14,15] induced by the decrease in the activity of cortical 5-HT neurons. Further experiments are in progress.

14) E.L. Way and C. Glasgow, "Psychopharmacology: A Generation of Progress," ed. by M.A. Lipton, A. DiMascio, and K.F. Killam, Raven Press, New York, 1978.
15) H. Kaneto, "No no Yakurigaku" ed. by H. Yoshida and K. Kuriyama, Ishiyaku Shuppan, Inc., Tokyo, 1975, pp. 121—142.

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Evidence for the Presence of O-Acetylserine in *Citrullus vulgaris*[1]

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O-Acetylserine (**2**), a substrate for cysteine synthase and β-substituted alanine synthase(s) in plants, was identified after transformation to N-acetylserine in the greenish-white epicarp and the reddish mesocarp of the intact fruits of water-melon (*Citrullus vulgaris*) in concentrations of 1.05×10^{-6}% and 0.98×10^{-7}%, respectively. No endogenous N-acetylserine was detected by the same procedure.

Keywords——amino acid; O-acetyl-L-serine; N-acetylserine; serine; β-substituted alanine; enzyme; β-substituted alanine synthase; *Citrullus vulgaris*; water-melon

Our recent reports have shown that O-acetyl-L-serine (**2**) has an important role as a key intermediate in the biosyntheses of β-substituted alanines, such as β-(pyrazol-1-yl)-L-alanine

1) This work was presented at Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, October, 1977, and was also briefly quoted in a reference cited in Ref. 4 of this paper.
2) Location: *1-33 Yayoi-cho, Chiba-shi, Chiba, 260, Japan.*