The Potent Depressant Effects of \( N^3 \)-Phenacyluridine in Mice

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The hypnotic activity of \( N^3 \)-phenacyluridine in 2.0 \( \mu \)mol/mouse by intracerebroventricular (i.c.v.) injection
was 20 times stronger than that of known \( N^3 \)-benzyluridine. In 0.5 \( \mu \)mol/mouse, i.c.v., this compound strongly
potentiated both pentobarbital- and diazepam-induced sleep as compared to \( N^3 \)-substituted uridines, including
\( N^3 \)-benzyluridine. Furthermore, the compound caused motor incoordination as well as decreasing spontaneous activity
in the same dose. These results indicate that among the \( N^3 \)-substituted uridines and related compounds previously
reported, \( N^3 \)-phenacyluridine possesses potent depressant effects.

Keywords \( N^3 \)-phenacyluridine; hypnotic activity; CNS depressant; uridine derivative

We previously reported the pharmacological effects of \( N^3 \)-substituted uridine, thymidine and 6-azauridine as
central nervous system (CNS) depressants.1–9 In these
connections, Yamamoto et al.1) found for the first time
that \( N^3 \)-benzyluridine (Fig. 1) exhibited hypnotic activity
in mice by intracerebroventricular (i.c.v.) injection.
In addition, uridine is known to have CNS depressant
effects such as a decrease in spontaneous activity in mice,10
anticonvulsant activity11) and a sleep promoting effect.12–14) This may suggest that pyrimidine nucleosides
play an important role in the CNS, while, the mechanism
is still unclear. For that reason, we have carried out a
structure–activity relationship study of \( N \)-substituted
pyrimidine nucleosides as CNS depressants.1–9) In
the present study, we successfully found a potent compound,
\( N^3 \)-phenacyluridine, which exhibited the strongest CNS
depressant effects among those previously reported.
Therefore, the pharmacological effects of \( N^3 \)-phenacyluridine were evaluated using mice.

MATERIALS AND METHODS

Animals Male ddN mice (Hokuriku Experimental Animals Laboratory, Kanazawa, Japan) weighing 23–28 g
were maintained at a constant temperature, eight to
a cage, with free access to food and water, in a room
automatically illuminated for 12 h and kept dark for 12 h.

Chemicals Uridine was purchased from Yamasa
Shoyu Co., Ltd. (Japan). Sodium pentobarbital and
diazepam were obtained from Tokyo Kasei Co., Ltd.
(Japan) and Yamanouchi Seiyaku Co., Ltd. (Japan),
respectively.

Preparation of \( N^3 \)-Substituted Uridine \( N^3 \)-Benzyluridine was prepared according to the method previously
reported.13) Similarly, \( N^3 \)-phenacyluridine was also prepared
with a slight modification. \( N^3 \)-Phenacyluridine:
mp 162–164°C; yield 63%; \( ^{1}H\)-NMR (DMSO-\( d_6 \)) \( \delta \): 3.06–3.40 (2H, m, 5'-H2), 3.90–4.35 (1H, m, 4'-H),
4.65–5.10 (2H, m, 2'-H, 3'-H), 5.45 (2H, s, NCH2),
5.93–6.11 (2H, m, 1'-H, 5'-H), 7.50–7.71 (5H, m, C6H5),
8.04 (1H, d, \( J=8 \) Hz, 6'-H); MS \( m/z \): 362 (M+).

Hypnotic Activity The compounds tested were sus-
pended in 3% Tween 80–saline solution because of their
insolubility in saline. The compounds (25 \( \mu \)l/mouse) were
given by i.c.v. administration according to the method of
Haley and McCormick16) and this method allowed
evaluation of the real CNS effect. The sleeping time pro-
duced by the compounds tested in mice was measured as
the time between the loss and recovery of the righting
reflex. For ascertaining the areas in the brain ventricular
system into which the drugs penetrated, India ink was
injected and the brains were sectioned. All injections
were successfully administered to the ventricle.

Effects of Uridine Derivatives on Pentobarbital-Induced
Sleep The compounds tested were injected, i.c.v., to mice
15 min before the pentobarbital challenge. Sodium pen-
tobarbital was administered intraperitoneally (i.p.)
to mice at a dose of 40 mg/kg. The pentobarbital-induced
sleeping time in mice was measured as described above in
hypnotic activity.

Effects of Uridine Derivatives on Diazepam-Induced
Sleep The compounds tested were injected, i.c.v., to mice
by 15 min before the diazepam challenge. Diazepam
was suspended in 1% Tween 80–saline solution and then
administered, i.p., to mice at a dose of 60 mg/kg. The
diazepam-induced sleeping time in mice was measured as
described above regarding hypnotic activity.

Motor Incoordination Motor incoordination was mea-

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Fig. 1. Structures of Uridine, \( N^3 \)-Phenacyluridine and \( N^3 \)-Benzyluridine.
sured by a bar test. After receiving the compounds, mice were placed on a bar (1 cm square, 40 cm height). Motor incoordination of the compound was expressed as the percent of mice that fell from the bar within 30 s.

Spontaneous Activity The spontaneous activity of mice was determined with an Animal Behavior Analyzer equipped with an NEC PC-9801 RX microcomputer (Muromachi Ind., Tokyo, Japan) after i.c.v. injection of the compounds tested. The treated mice were placed in the center of a 30 x 30 cm square plastic box. Measurement of the activity was evaluated as the total distance (cm) traveled by the animals within 1 h after the injection.

Statistical Analysis An ANOVA was performed and multiple comparisons of the means were evaluated by the Bonferroni test.

RESULTS

Hypnotic Activity The hypnotic activity of N3-phenacyluridine was evaluated and compared with that of N3-benzyluridine previously reported11 (Fig. 2). Both compounds possessed hypnotic activities in a dose-dependent manner by i.c.v. injection. In contrast to N3-benzyluridine, which failed to induce hypnotic activity at 1.0 μmol/mouse, N3-phenacyluridine induced a sleeping time of 220 min in mice at the same dose. At a dose of 2.0 μmol/mouse, the sleeping time induced by N3-phenacyluridine was 570 min, 20-fold longer than that of N3-benzyluridine.

Potentiation of Pentobarbital-Induced Sleep by Uridine Derivatives Figure 3 shows the effects of N3-phenacyluridine and N3-benzyluridine on pentobarbital-induced sleep. The sleeping time of the control, N3-phenacyluridine and N3-benzyluridine administered to mice with sodium pentobarbital were 40, 120 and 229 min, respectively. Although 0.5 μmol/mouse of N3-phenacyluridine did not produce hypnotic activity in mice, the compound at the same dose significantly prolonged the pentobarbital-induced sleeping time as compared to the control and the N3-benzyluridine treated groups. N3-Benzyluridine tended to potentiate pentobarbital-induced sleep, but the effect was not significant with the same dose.

Potentiation of Diazepam-Induced Sleep by Uridine Derivatives Figure 4 shows the effects of N3-phenacyluridine and N3-benzyluridine on diazepam-induced sleep. The sleeping times of the control, N3-phenacyluridine and N3-benzyluridine administered to mice with diazepam were 31, 103 and 2124 min, respectively. N3-Phenacyluridine (0.5 μmol/mouse) significantly prolonged the diazepam-induced sleeping time as compared to the control and the N3-benzyluridine treated groups.

Motor Incoordination As shown in Fig. 5, N3-phenacyluridine and N3-benzyluridine (0.5 μmol/mouse, i.e.v.) exhibited motor incoordination in mice. Recovery time from the motor incoordination caused by N3-phenacyluridine and N3-benzyluridine was 7 and 6 h, respectively.
**Spontaneous Activity** The spontaneous activity of mice treated with N<sup>3</sup>-phenacyluridine and N<sup>3</sup>-benzyluridine (0.5 μmol/mouse) is summarized in Table I. The spontaneous activity of mice treated with N<sup>3</sup>-phenacyluridine was significantly reduced to 15% of the control level. Although N<sup>3</sup>-benzyluridine also reduced spontaneous activity, it was not significantly different from the control level.

**DISCUSSION**

We had reported for the first time the structure–activity relationships of N<sup>3</sup>-substituted uridine, thymidine and 6-azauridine on CNS depressant activity in mice. 1–9 N<sup>3</sup>-Benzy1 substituted uridine and its related derivatives, i.e., N<sup>3</sup>-o, m or p-xylyluridine produced 36–72 min of sleeping time in mice after i.c.v. injection at 2.0 μmol/mouse, suggesting that aromatic groups at the N<sup>3</sup> position on uridine play an important role in inducing the CNS activity. 41 In the present study, we further designed a novel derivative, N<sup>3</sup>-phenacyluridine, which also has a benzyl related group at the N<sup>3</sup> position of uridine. N<sup>3</sup>-Benzyluridine exhibited strong CNS depressant effects, e.g., the potentiation of pentobarbital-induced sleep, motor incoordination and a decrease in spontaneous activity after i.c.v. injection with 1.0 μmol/mouse in mice. 1,3,4 Although dose of 0.5 μmol/mouse of N<sup>3</sup>-benzyluridine did not cause significant effects on the CNS, the same dose of N<sup>3</sup>-phenacyluridine examined here exhibited marked effects in all pharmacological indices. Therefore, we have successfully found that N<sup>3</sup>-phenacyluridine has the most potent hypnotic activity among the derivatives examined so far.

The benzodiazepine receptor is known to be one of the components of the γ-aminobutyric acid (GABA<sub>4</sub>) receptor, which forms a chloride channel in the CNS. 18 Since the degree of the potentiation of diazepam-induced sleep was markedly different between the N<sup>3</sup>-phenacyluridine and N<sup>3</sup>-benzyluridine, one possible explanation for our results is that N<sup>3</sup>-phenacyluridine may affect the receptor complex. Studies of the receptor assay on the interaction of N<sup>3</sup>-phenacyluridine with a GABA–benzodiazepine receptor–chloride channel complex are now in progress.

In conclusion, the present study strongly confirms our previous findings that N<sup>3</sup>-substituted nucleosides have hypnotic and sedative activities, and that the introduction of an aromatic group such as a benzyl and/or phenacyl group onto the N<sup>3</sup>-position is an important factor in eliciting the central depressant effects of nucleoside derivatives.

**REFERENCES**