COMPARISON OF LOCAL ANESTHETIC ACTIVITIES BETWEEN OPTICAL ISOMERS OF cis-1-BENZOYLOXY-2-DIMETHYLAMINO-1,2,3,4-TETRAHYDRONAPHTHALENE

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Abstract—The optical isomers of cis-1-benzoyloxy-2-dimethylamino-1,2,3,4-tetrahydrophenanthrene (YAU-17) were compared for their local anesthetic activity, acute toxicity, spasmyloytic activity, and partition coefficient between chloroform and phosphate buffer. 1-YAU-17 was more active than d-YAU-17 in blocking the conduction of action potentials in isolated frog sciatic nerves. The difference in local anesthetic activities between the optical isomers was further substantiated by in vivo tests for corneal anesthesia, intracutaneous anesthesia and sciatic nerve block in guinea-pigs. Similarly, the i.v. injection to mice revealed a higher toxicity for 1-YAU-17 as compared to its d-isomer. In these tests, the potency ratios of the enantiomers ranged from 2 to 4, and the racemate had an intermediate potency. On the contrary, no difference among the compounds was found in their liposolubility, partition coefficient, and spasmyloytic activity examined with isolated guinea-pig ileum. These results indicate that the steric factors play an important role in the production of different local anesthetic activities between the optical isomers of YAU-17, and their local anesthetic potency tends to be correlated to their intravenous acute toxicity but not to their spasmyloytic activity.

Stereoselectivity of local anesthetics, as defined by the difference in local anesthetic activities between stereoisomers, has been investigated by several authors (1–3). In experiments on optical isomers of prilocaine, Akerman et al. stated that the differences in their local anesthetic activities were found in vivo but not in vitro (1), and this was ascribed to better localization at the site of application of the more active isomer with the aid of biochemical techniques (2). It was recently reported that local anesthetic activities of S(−)-HS37 were consistently more potent than those of its optical isomer in both in vitro and in vivo testing procedures (3). In addition, the i.v. acute toxicity of the enantiomers was parallel with their local anesthetic activity in accordance with the findings of Luduena (4) that there was a high degree of correlation between the anesthetic potency and the systemic toxicity of local anesthetic drugs (3).

The present author has already reported that local anesthetic activity of dl-1-arylxyloxy-2-dimethylaminocycloalkane derivatives including racemic YAU-17 was positively correlated to i.v. acute toxicity in mice but not to spasmyloytic activity.
potency in isolated guinea-pig ileum (5). It was also demonstrated that racemic YAU-17
and its trans isomer, which were more potent than procaine and lidocaine, differed in
their local anesthetic activities (6). Furthermore, the recent separation of racemic YAU-17
into optical isomers made it possible to investigate the role of steric factors in the local
anesthetic activity.

The present report describes the difference in local anesthetic activities between the
eanitiomers of YAU-17 in vitro and in vivo. Their i.v. acute toxicity, spasmolytic activity
and distribution coefficient in a lipid-water system were also studied. The chemical structure
of YAU-17 is shown in Fig. 1.

MATERIALS AND METHODS

Inhibition of evoked action potential in isolated nerve

The sciatic nerve-gastrocnemius muscle preparation isolated from frogs (Rana nigromaculata) was immersed in a chamber with 5 compartments containing 2 to 10 ml of frog
Ringer's solution (pH 7.4) at room temperature (23–25°C). Electrical stimulations of the
nerve and records of action potentials were made through a pair of Ag-AgCl electrodes con
nected to the bathing medium in both sides of the chamber. Following stimulations of the
proximal end of the nerve by rectangular pulses (0.1 cps, 0.05 msec duration, submaximal
voltage), the action potentials in the distal end were recorded on an oscilloscope (Sanei Sokki
Instrument, BO-207). The blocking effect of drugs on the conductivity was calculated from
the amplitude of action potentials measured before and 30 min after the addition of drugs
to the middle compartment, and a 50 °; inhibitory dose (1D50) was determined from dose-
response curves for comparison of the activity. Five preparations were used for each dose
of local anesthetics.

Local anesthetic activity in guinea-pigs

Local anesthetic tests were performed using 6 to 8 male guinea-pigs weighing 300 to
500 g for each dose level.

According to the method of Chance and Lobstein (7), an anesthetic solution (0.1 ml)
was instilled into the conjunctival sac and left for 1 min. The cornea was stimulated 5 times
with a test hair 5 min after application of the anesthetic solution. Then, the test of 5 stimuli
was performed every 5 min and the number of stimuli which did not cause the corneal reflexes
was counted during 30 min. The mean dose representing a 50 °; inhibition of the reflexes
(ID50) was determined from a dose-response curve in which the inhibitory percentage was
plotted against log concentrations of the drug. The significance of difference in percent
inhibition in the same doses was calculated according to Student's t-test.

For the measurement of intracutaneous anesthetic activity (8), 0.2 ml of a drug solution
was injected intracutaneously into shaven backs of the animals, and ID50 values were
estimated in the same way as mentioned above for the corneal test.

According to the method of Shackell (9), 0.3 ml of test solution was injected close to
the sciatic nerve at the hip of guinea-pigs, and the motor paralysis of hindlegs described by
Loomis and Spielmeyer (10) was observed to measure onset and duration of the sciatic nerve
block. Duration of the action was determined by the last of positive readings tested every 5 min.

*Acute toxicity in mice*

The drugs were injected i.v. to male mice of ICR-SLC strain weighing 28 to 35 g at a rate of 0.1 ml/10 g/10 sec, and the animals were kept under observation for 7 days. LD50 values and their confidential limits were determined by the method of Litchfield and Wilcoxon using 5 groups of 10 aggregated animals.

*Spasmolytic activity in isolated ileum*

Activities of test compounds to antagonize acetylcholine (5 \times 10^{-7} M) and barium chloride (10^{-3} M) were assessed with ileal strips from guinea-pigs suspended in 100 ml of oxygenated Tyrode's solution (pH 7.8) at 30 °C. The drugs were added 10 min before treatment with the agonists and ED50 values were determined using 6 preparations for each drug.

*Partition coefficient between chloroform and phosphate buffer*

After each drug (3 μmoles), which was dissolved in 20 ml of 0.1 M sodium phosphate buffer at pH 6.0 and 7.0, was shaken vigorously with an equal volume of chloroform for 10 min (11), the amounts of the compound distributed in both phases were determined by the UV spectrophotometric method. Following the evaporation of chloroform under reduced pressure, the drug was dissolved in 0.1 N HCl solution. The optical density of the solution and the buffer phase was measured at 235 mμ with a spectrophotometer (Hitachi Perkin-Elmer, Model 139).

*Drugs*

Drugs used were as follows: 1-, d- and dl-cis-1-benzoyloxy-2-dimethylamino-1,2,3,4-tetrahydronaphthalene (YAU-17) hydrochloride, acetylcholine chloride (Daiichi Seiyaku), barium chloride (Kokusan Chemical) and papaverine hydrochloride (Torii Co.). All drugs were freshly dissolved in 0.9% saline at indicated concentrations or doses which were expressed in terms of the respective chemical forms mentioned above. For local anesthetic tests, the pH of drug solutions was adjusted to 6.0.

Synthesis of racemic YAU-17 and its separation into optical isomers were achieved by Hirata et al. of Organic Chemical Research Department, Yamanouchi Pharmaceutical Co. The configurations of the enantiomers were (−)-(1R, 2S)-YAU-17 (1-YAU-17) and (−)-(1S, 2R)-YAU-17 (d-YAU-17).

**RESULTS**

*Inhibition of evoked action potential in isolated nerve*

Optical isomers of YAU-17 and the racemic mixture were compared for their ability to block the conduction of action potentials in sciatic nerve preparations taken from frogs. As shown in Fig. 2, the conductivity was found to be suppressed dose-dependently after addition of drugs in concentrations of 10^{-3} to 10^{-6} M (0.03-0.3 μg), and the difference in the conduction block between the enantiomers was statistically significant (p < 0.05). ID50 values for 1-, dl- and d-YAU-17 were 2.0 \times 10^{-5}, 2.6 \times 10^{-3} and 8.5 \times 10^{-3} M, respectively,
indicating the potency ratio of 1-, dl- and d-YAU-17 was approx. 4:3:1.

Local anesthetic activity in guinea-pigs

Racemic YAU-17 and its optical isomers produced a similar pattern in the time course of corneal block, although their doses exerting an equipotent corneal anesthetic action were not the same for each other. In each fixed dose level used, 1-YAU-17 induced a noticeably longer duration of the corneal block than the d-isomer, and the racemate was intermediate between the optical isomers in this respect (Fig. 3A). Additionally 1- and dl-YAU-17 were significantly more potent than the d-form in reducing the total frequency of corneal reflexes during 30 min as presented in Fig. 4A. The potency ratio of 1-, dl- and d-YAU-17, which was estimated by ID50 values in Table 1, was approx. 4:3:1.

A higher activity for 1-YAU-17 as compared to the d-isomer was further substantiated in the intracutaneous assay and the difference in their anesthetic activities was statistically significant (p<0.01). More precisely, the results obtained from the intradermal wheal were similar to those from the corneal...
Fig. 4. Corneal (A) and intracutaneous (B) anesthetic activities of 1-, d- and dl-YAU-17 in guinea-pigs. Percent inhibition was determined by local anesthetic effect during the first 30 min. Each mark shows mean ± S.E. from 8 animals. * p<0.05, ** p<0.01: significantly differed from the values of d-YAU-17. Notations are as in Figs. 2 and 3.

Table 1. Local anesthetic activities (ID50, % concentration) and acute toxicities of 1-, d- and dl-YAU-17

| Compound    | Corneal anesthetic (%) | Intracutaneous anesthetic (%) | Acute toxicity LD50 (mg/kg i.v.) |
|-------------|------------------------|-------------------------------|---------------------------------|
| l-YAU-17    | 0.05                   | 0.02                          | 12.4 (11.5-13.3)                |
| d-YAU-17    | 0.2                    | 0.08                          | 26.9 (24.5-29.0)                |
| dl-YAU-17   | 0.07                   | 0.03                          | 17.9 (16.1-19.9)                |

ID50 values were determined by local anesthetic effect during the first 30 min using 8 guinea-pigs for each dose and LD50 values (95% confidential limits) were measured by employing 10 mice per dose.

method, with respect to the time course of action and the potency ratios among the optical isomers and racemate. However, the effective concentrations of the drugs in the intracutaneous test were lower than those required for the corneal anesthesia (Fig. 3 and 4, Table 1).

Additional experiments were directed toward confirming the difference in local anesthetic activities between the enantiomers of YAU-17. As demonstrated in Fig. 5, injection of the compounds close to the sciatic nerve resulted in a dose-related motor paralysis of the hindleg. 1-YAU-17 was 2.5 to 5.5 times as potent as the d-isomer to produce a block of the same duration, and racemic YAU-17 was intermediate between the optical isomers.
in this regard. On the other hand, all compounds exhibited the nerve block within 1 min, showing no difference in the onset of action irrespective of their definite difference in the duration of action.

Acute toxicity in mice

Acute toxicity of racemic YAU-17 and its optical isomers was determined in mice. After i.v. injection of lethal doses of the compounds, death due to respiratory arrest occurred in most animals within 20 min.

It was evident from LD50 values in Table 1 that the toxicity relationship for 1-, dl- and d-YAU-17 was 2.2:1.5:1. Thus, their rank order of potency in the systemic toxicity remained the same as was established by the local anesthetic tests.

Spasmylytic activity in isolated ileum

Antagonistic activities of racemic YAU-17 and its optical isomers against acetylcholine and barium chloride were evaluated with ileal strips from guinea-pigs. As shown in Table 2, the drugs suppressed the effects of the spasmogenic substances and their activity was almost comparable to that of papaverine. No difference in spasmylytic activity of the isomers was detected, contrary to the results in local anesthetic and acute toxicity tests.

Partition coefficient between chloroform and phosphate buffer

All compounds of YAU-17 transferred quantitatively from an aqueous phase into a lipid phase, when distributed between chloroform and 0.1 M phosphate buffer at pH 7.0.

| Table 2. Antagonistic activities (ED50, M) of 1-, d- and dl-YAU-17 and papaverine against acetylcholine (ACh) and barium chloride in ileal strips from guinea-pigs |
|-----------------------------------------------|
| Compound          | ACh (5 × 10⁻⁷ M) | BaCl₂ (10⁻³ M) |
|-------------------|------------------|----------------|
| 1-YAU-17          | (3.9±0.4)×10⁻³   | (3.5±0.5)×10⁻³ |
| d-YAU-17          | (3.2±0.3)×10⁻³   | (2.5±0.4)×10⁻³ |
| dl-YAU-17         | (3.6±0.6)×10⁻³   | (2.1±0.6)×10⁻³ |
| Papaverine        | (3.0±0.3)×10⁻³   | (1.8±0.3)×10⁻³ |

ED50 values (M) represent mean±S.E. of 6 experiments.

| Table 3. Partition coefficients of 1-, d- and dl-YAU-17 between chloroform and phosphate buffer |
|-----------------------------------------------|
| Compound          | Amounts of compounds (10 nmoles) | Partition Coefficient |
|                   | Chloroform | Phosphate buffer |               |
|-------------------|------------|------------------|---------------------|
| 1-YAU-17          | 296±2      | 1.3±0.3          | 255±38              |
| d-YAU-17          | 269±2      | 1.4±0.5          | 286±79              |
| dl-YAU-17         | 288±3      | 1.4±0.4          | 257±64              |

Each compound (3 nmoles) was partitioned between equal volumes (20 ml) of chloroform and 0.1 M phosphate buffer (pH 7.0). The values show mean±S.E. from 5 determinations. a, amounts of compound in chloroform/amounts of compound in phosphate buffer
Partition coefficients of the drugs were 250 or more, with no significant difference among them (Table 3). Similar results were obtained in the case of the buffer at pH 6.0.

DISCUSSION

Present studies demonstrated that the optical isomers of YAU-17 produced significant differences in their local anesthetic potencies both in vitro and in vivo. These results were in good agreement with those reported on the optical isomers of spirosuccinimides (12) and carbanilic acid esters (HS37) (3), both of which have been considered to possess stereoselectivity in their local anesthetic activities. In recent studies on the spirosuccinimides Akerman described that, although the isomers differed in the excitation block of isolated nerves, the uptake of the compounds by isolated nerves was similar, suggesting that their effects on the nerve membrane are intrinsically different (13). On the other hand, the enantiomers of prilocaine were equally effective in preventing the impulse propagation in the isolated nerves, despite their different local anesthetic activity in situ (1). This was explained by the variance in local concentrations of enantiomorphous prilocaines at the site of application (2, 12).

As shown in Fig. 2, the optical isomers of YAU-17 differed in blocking the conduction of action potentials in the isolated nerves, contrary to the results with the enantiomers of prilocaine (1). Furthermore, the partition coefficients which are important for local anesthetics to penetrate the lipid membrane (14) were similar between the enantiomers of YAU-17, suggesting that they reach their site of action in equal concentrations. The steric factors may therefore be considered to be responsible for the observed differences in local anesthetic activities between the optical isomers of YAU-17.

Racemic YAU-17 was also proved to have a high liposolubility, which may contribute to the higher anesthetic activity of racemic YAU-17, especially in corneal block (6), as compared to that of procaine with comparatively low liposolubility (11).

As expected from the previous findings that the local anesthetic activity was closely correlated to i.v. acute toxicity (4, 5), 1-YAU-17 with higher local anesthetic potency was more toxic than its d-isomer in mice. In this regard, the systemic toxicity of enantiomers of HS37 changed in parallel with their local anesthetic activity (3), whereas no difference of the toxicity was found between enantiomers of prilocaine which were stereospecifically indistinguishable from each other in the local anesthetic activity (2, 3). Therefore, it may be plausible that positive correlation between local anesthetic potency and i.v. acute toxicity (4) holds true for optical isomers of local anesthetics. In contrast, spasmolytic activity of optically active YAU-17 showed no relationship either to local anesthetic potency or to systemic toxicity. These results coincided with those obtained from dl-1-aroyloxy-2-dimethylaminocycloalkane derivatives including racemic YAU-17 (5).

The stereoselectivity of local anesthetics, which was shown in previous studies (3, 12, 13, 14) was confirmed in the present experiments and evaluated to be of minor importance for the intensification of activities in comparison with the case of other stereospecific drugs as are already known (14). For instance, (−)-propranolol was 100 times as potent as its
(±)-isomer in preventing the positive inotropic action induced by isoprenaline (15), whereas the ratio of potency of 1-YAU-17 to that of d-YAU-17 was 4 in local anesthetic tests. Accordingly, it is concluded that the isomers of YAU-17 differed definitely in their local anesthetic activities unlike optical isomers of prilocaine (1) or lotucaine (16), although the extent of difference in the anesthetic activity between the enantiomers of YAU-17 was not so prominent.

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