COMPARISON OF LOCAL ANESTHETIC ACTIVITIES BETWEEN CIS AND TRANS ISOMERS OF DL-1-BENZOYLOXY-2-DIMETHYLAMINO-1,2,3,4-TETRAHYDRONAPHTHALENE

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Abstract—The local anesthetic activities of cis- and trans- dl-1-benzoyloxy-2-dimethylamino-1,2,3,4-tetrahydronaphthalene were compared using several methods with guinea-pigs. The cis compound (YAU-17) was 2.9 to 6 times as potent as its trans isomer and exceeded procaine, lidocaine and cocaine in potencies of corneal anesthesia, intracutaneous anesthesia and sciatic nerve block. In another experiment on isolated frog sartorius muscle, all of the local anesthetics suppressed the twitch contractions elicited by stimulation of the sciatic nerve. The neuromuscular blocking activity of the cis compound was more pronounced than that of the trans form. Supersensitivity to noradrenaline was produced by both of the stereoisomers in isolated vas deferens of guinea-pigs although there was no difference in the activity. The sensitizing action was also demonstrable with cocaine and lidocaine but not with procaine. When injected intravenously into mice, the cis compound was twice as toxic as its isomer. It is postulated that stereoselectivity is to some extent involved in the mechanisms of action of the local anesthetic agents.

Since the classical investigations on local anesthetic properties of naturally occurring cocaine, many useful local anesthetics have been introduced in the field of clinical surgery and dentistry. This in turn has prompted intensive investigations into their structure-activity relations, mechanisms of action and physicochemical properties (1, 2).

On the other hand, the stereospecificity of local anesthetics has been considered to be only of minor importance because slight or no difference in the anesthetic activity was found between stereoisomers unlike other stereospecific drugs such as parasympathomimetics and $\beta$-adrenergic blocking agents (2, 3, 4). In this regard, Schönenberger et al. reported that aminoacyl ephedrines showed distinct differences in local anesthetic activity between stereoisomers (5). The stereoselectivity has also been studied by Åkerman et al. (6, 7).

In order to investigate the role of steric factors in local anesthetic activity, the new cis and trans isomers, the chemical structure of which is shown in Fig. 1, were used for the present work. Since the effects of certain local anesthetics on neuromuscular transmission and on the response of vas deferens to noradrenaline

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**Fig. 1.** Chemical structure of cis- and trans-dl-1-benzoyloxy-2-dimethylamino-1, 2, 3, 4-tetrahydronaphthalene.
were already examined (1, 8), similar experiments with the cis and trans isomers were also performed.

MATERIALS AND METHODS

Local anesthesia

Groups of 6 to 8 male guinea-pigs weighing 300 to 500 g were used for the local anesthetic tests.

The cornea was stimulated five times with a fine mandrel of needle for injection, 5 min after application of the anesthetic solution into the conjunctival sac for one min. The test of the five stimuli was applied every 5 min for 30 min and the number of stimuli which did not cause the corneal reflex was counted (9). The dose representing a 50% inhibition of the reflex (ID50) was determined from the dose-response curves in which the inhibitory percentage of the reflex was plotted against the logarithm of each concentration of a drug, and used for comparison of the activity.

For the wheal method (10), 0.2 ml of the test solution was injected intracutaneously on the shaven backs of the animals and ID50 values were estimated by the same manner as mentioned above for the corneal anesthesia.

Another test on sciatic nerve block was carried out by injecting 0.3 ml of the test solution close to sciatic nerve at the hip (11) and the motor paralysis of the hindleg described by Loomis and Spielmeyer (12) was observed to measure duration of the action which was determined by the last of positive readings tested every 5 min.

Neuromuscular block

Sciatic nerve-sartorius muscle isolated from the frog (Rana nigromaculata) was suspended in an organ bath containing 100 ml of frog Ringer solution (pH 7.4) gassed with a mixture of 95% O2 and 5% CO2 at 20°C. The stimulator (Nihon Kohden, MSE-3) set to deliver rectangular pulses (0.1 cps, 0.5 msec duration, submaximal voltage) was used for stimulation of the nerve. The twitch contractions were recorded through a strain gauge (Nihon Kohden, SB-1T) on a polygraph (Nihon Kohden, RM-150). The percent inhibition by drugs was calculated from the difference in height of contractions between before and 10 min after the addition of drugs. For each drug, 4 to 5 preparations were used.

Supersensitivity to noradrenaline

The vas deferens isolated from guinea-pigs was mounted in 100 ml of oxygenated Tyrode solution (pH 7.8) at 36°C. The recordings of contraction were made on a polygraph as mentioned above for neuromuscular block. The tissues were exposed to test drugs 5 min before treatment with noradrenaline (10^{-3} M) and the drug activity was expressed as mean percent increase in the contractility obtained from 5 preparations.

Acute toxicity

The drug was intravenously injected into male mice (28~35 g) at a constant rate of 0.1 ml/10 g/10 sec. The LD50 and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon using 6 groups of 10 aggregated animals.
Drugs

Drugs used were as follows: cis- and trans- dl-1-benzoyloxy-2-dimethylamino-1,2,3,4-tetrahydronaphthalene hydrochloride, procaine hydrochloride (Sanko Seiyaku), lidocaine hydrochloride (Fujisawa Co.), cocaine hydrochloride (Tanabe Seiyaku), d-tubocurarine chloride (Tokyo Kasei) and noradrenaline hydrochloride (Sankyo Co.).

The cis and trans isomers were prepared at the Research Laboratories, Yamanouchi Pharmaceutical Co. and the cis compound was coded YAU-17. All drugs were dissolved in physiological saline at desired concentrations and the pH's of the solutions were 5.5 to 6.0. Concentrations or doses were expressed in the terms of the respective chemical forms mentioned above.

RESULTS

Local anesthesia

Comparison of local anesthetic activity between the cis compound (YAU-17) and its trans isomer was made by employing several methods in guinea-pigs.

Corneal anesthesia was produced dose-dependently by application of the stereoisomers and a statistically significant difference was found in their activity (Fig. 2). The ID50 values of the cis and the trans compound were 0.07 and 0.2%, respectively, indicating the cis compound was 2.9 times as active as the trans isomer. Corneal block was also exhibited after treatment with procaine, lidocaine and cocaine. However, the activities of these reference drugs were less potent than those of the stereoisomers.

In the intracutaneous wheal, the isomers caused a high degree of local anesthesia (Fig. 2). The ID50 values of the cis and the trans substance were 0.03 and 0.12%, respectively, indicating the cis compound was 4 times as potent as the trans isomer. The difference in their activity was also highly significant as shown in Fig. 2. Additionally, the wheal test was much more sensitive for local anesthetics than the corneal method. This was especially evident with procaine and lidocaine.

![Fig. 2. Corneal and intracutaneous anesthetic activities of stereoisomers and other drugs in guinea-pigs. Percent inhibition was determined by anesthetic effect during the first 30 min. Each point represents mean±S.E. from 8 animals. Cis and Trans: cis- and trans- dl-1-benzoyloxy-2-dimethylamino-1,2,3,4-tetrahydronaphthalene, respectively, P: procaine, L: lidocaine, C: cocaine. *p<0.05, **p<0.01: significantly differed from the values of the trans compound with Student’s t-test.](image-url)
Another experiment on sciatic nerve block was carried out in which the isomers exerted motor paralysis in a dose-related manner. The isomers significantly differed in their activity and the cis compound was 5 to 7 times as potent as the trans form in order to produce a block of the same duration (Fig. 3). Similarly, the sciatic nerve block was induced by the reference drugs.

Neuromuscular block

Sciatic nerve-sartorius muscle preparations isolated from frogs were used in order to examine the effect of drugs possessing local anesthetic activity on neuromuscular transmission. The cis and trans isomers caused a marked depression of indirectly elicited twitches without causing a transient initial phase of contractile potentiation. The neuromuscular block was also induced by treatment with either procaine or lidocaine as shown in Fig. 4. The cis form was more potent than the trans isomer which was almost equipotent to procaine and lidocaine in the neuromuscular blocking activity (Fig. 5). The fact that the activity of d-tubocurarine was more potent than that of the other drugs is also worthy of mention.

![Fig. 3. Sciatic nerve block of stereoisomers and other drugs in guinea-pigs. Each point represents mean ± S.E. from 6 animals. Abbreviations are as in Fig. 2.](image1)

![Fig. 4. Effect of stereoisomers and other drugs on response of isolated frog sartorius muscle to indirect stimulation. d-Tc: d-tubocurarine. Abbreviations are as in Fig. 2.](image2)

![Fig. 5. Effect of stereoisomers and other drugs on response of isolated frog sartorius muscle to indirect stimulation. Percent inhibition was calculated from the amplitude of contractions before and 10 min after addition of drugs. Each point represents mean ± S.E. ( ) : number of experiments, d-Tc: d-tubocurarine. Abbreviations are as in Fig. 2.](image3)
Supersensitivity to noradrenaline

The cis and trans isomers potentiated the contractile response of isolated guinea-pig vas deferens to noradrenaline (10⁻⁵ M) and little difference was detected between their supersensitizing activity. The supersensitivity to noradrenaline was also demonstrable with cocaine and lidocaine but not with procaine. Cocaine markedly exceeded the other drugs in activity (Fig. 6).

Acute toxicity

LD50 values (95% confidential limits) of the cis and the trans compound were 17.9 (16.1~19.9) and 36.5 (33.0~40.3) mg/kg i.v., respectively, in mice. From the LD50 values, the cis compound was twice as toxic as the trans isomer. Characteristic symptoms of lethal doses included incoordination, convulsion and respiratory arrest. Death occurred in most animals within 30 min after injection of the isomers.

DISCUSSION

The stereoselectivity of local anesthetics was studied by using the new cis and trans isomers in which the benzoyloxy and the dimethylamino group were structurally semi-rigid in the molecules and it was found that the cis and trans isomers exhibited highly significant differences in their local anesthetic activities, with the cis compound being 2 to 6 times as potent as its trans isomer. Beneš et al. reported that in cyclohexylesters of substituted alkoxycarbonilid acids, local anesthetic activity of trans compounds was more potent than that of the corresponding cis isomers (13). Similarly, Borne et al. stated that the most active conformer had a trans configuration in 2-azabicyclo [2, 2, 2] octane derivatives (14). On the contrary, the cis compound produced more potent local anesthetic activity than the trans isomer in the present study. Although the discrepancy regarding the favourable configuration for local anesthetic activity remains unsolved, it may be speculated that some degree of stereoselectivity is involved in the action of cis and trans isomers. On the other hand, Åkerman reported that R(+)·HS37 was more effective than its optical isomer in local anesthetic activity with the relative potency between 2 and 3 (7). Accordingly, it is concluded that the degree of stereospecificity of local anesthetics is much lower than that reported on
parsyspathomimetics or \(\beta\)-adrenergic blocking agents (2, 3, 4).

When injected intravenously into mice, the cis compound (YAU-17) was more toxic than its trans isomer. This result was in good agreement with the previous finding that local anesthetic activity was positively correlated to intravenous acute toxicity (15). In this connection, it was reported that optical isomers differing in local anesthetic activity produced definite differences in intravenous acute toxicity (7). Thus, \(R(+)\)-HS37 was more toxic than \(R(-)\)-HS37 (7), whereas no difference was found in the acute toxicity between optical isomers of prilocaine or mepivacaine (7, 16).

It is well known that procaine has a blocking action at the neuromuscular junction of skeletal muscle without any action on the response to direct stimulation (1). Furukawa speculated that both lowered reactivity of end-plate to acetylcholine and decrease in the release of acetylcholine from the nerve endings were important factors for the neuromuscular block elicited by procaine (17). It was also described by Steinbach that the neuromuscular blocking action of lidocaine seemed unrelated to its local anesthetic action (18). The neuromuscular blocking action of procaine and lidocaine was confirmed in the present work. Similarly, the cis and trans isomers blocked neuromuscular transmission and the cis compound was found to be more active than the other local anesthetics. Therefore it may be plausible that neuromuscular blocking activity of local anesthetics in isolated sartorius muscle of frogs is related to stabilization of neural membrane. Further study of the effects of local anesthetics on neuromuscular transmission will be reported in a forthcoming paper.

Numerous reports have shown that cocaine exhibits a potentiation in the response of smooth muscle to catecholamines, and such is presumed to result from the inhibition of catecholamine uptake into sympathetic nerve endings (19, 20). It has been proposed, however, that the potentiation induced by cocaine involves a postsynaptic component (8, 21). In the present experiment, the supersensitivity of the isolated vas deferens to noradrenaline was produced by local anesthetics, except for procaine. The most prominent enhancement of the contraction was elicited by cocaine, whereas little difference was detected in the sensitizing activity between the cis and trans isomers. These results suggest that the supersensitizing activity of local anesthetics does not parallel the local anesthetic potency.

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