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Effect of 9-Amino-2,3,5,6,7,8-Hexahydro-1H-Cyclopenta-(b)-Quinoline Monohydrate Hydrochloride (NIK-247) on Cholinergic Enzyme Activity in Rats

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SHIBANOKI, S., Y. ISHII, T. KUBO, M. KOGURE, S. ASAI AND K. ISHIKAWA. *Effect of 9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta-(b)-quinoline monohydrate hydrochloride (NIK-247) on cholinergic enzyme activity in rats.* PHARMACOL BIOCHEM BEHAV **39**(2) 499–502, 1991.—An in vitro comparison demonstrated that the concentration of NIK-247 that inhibited cholinesterase (ChE) activities to half the normal level (ID_{50}) was 1.3×10^{-6} M. This value was higher than those for both physostigmine (PHY; 1.2×10^{-7} M) and tetrahydroaminoacridine (THA; 3.6×10^{-7} M), which are used as cholinesterase inhibitors in the treatment of cholinergic deficits. Neither NIK-247 nor THA affected the activity of choline acetyltransferase (ChAT). These inhibitions of ChE by NIK-247 and PHY lasted for 2 h, while that by THA lasted for over 4 h. In the effects of NIK-247 and PHY, the concentrations of intrastriatal acetylcholine (ACh) were changed in relation to the inhibition of the ChE activity. However, THA caused a transient increase in the ACh level lasting for only 2 h instead of inhibiting the enzyme activity for over 4 h. These findings suggest that NIK-247 is a drug with a similar profile in its effect on cholinergic neurons to PHY, the prototype drug among ChE inhibitors. The data indicate that NIK-247 may be useful as a drug for the treatment of central as well as peripheral deficits of the cholinergic mechanism.

9-Amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta-(b)-quinoline monohydrate hydrochloride (NIK-247)
Centrally acting cholinesterase inhibitor Rats Physostigmine Tetrahydroaminoacridine

9-Amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta-(b)-quinoline monohydrate hydrochloride (NIK-247) is a structural analogue of tetrahydroaminoacridine (THA). It has been established that THA is a centrally acting potent inhibitor of cholinesterase (ChE), an enzyme degrading acetylcholine (ACh), based on in vivo and in vitro experiments (4,6). NIK-247 is assumed to have similar pharmacological properties including an effect on ChE from the standpoint of the structure-activity relationships. It has been reported that drastic degeneration of cholinergic neurons is observed in the basal nucleus in Alzheimer-type senile dementia (14). Other reports demonstrated biochemically the occurrence of decreases in cholinergic markers, i.e., the activities of ChE and choline acetyltransferase [ChAT; see (1)] and the concentration of this neurotransmitter in the brain (12). Since ChE inhibitors are expected to increase the concentration of ACh, such inhibitors have been proposed to be effective in the treatment of mental disorders including senile dementia [for review, see (10)]. These substances include physostigmine (PHY), the prototype of this drug group, and THA. Thus NIK-247 has also aroused interest for possible application in such disorders, and behavioral effects on passive avoidance response in mice (11) as well as attenuation of learning deficit in pallidus-lesioned rats have been described (13). However, little effect has yet been reported regarding the biochemical profile of this compound. The present study was carried out to examine the effect of NIK-247 on the activities of enzymes related to cholinergic metabolism. The in vivo effect of NIK-247 on the activity of ChE and the concentration of ACh in the striatum was also examined. For comparison, the effects of PHY as well as of THA were studied.

METHOD

Animals

Wistar rats, each weighing about 250 g, were used throughout the experiments. They were obtained from Shizuoka Experimental Animal Colony (Hamamatsu, Japan) and housed for at

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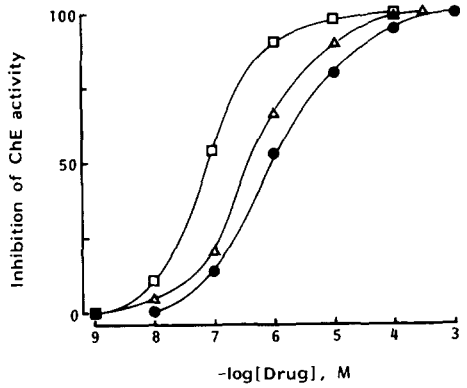

FIG. 1. Effects of NIK-247 (●), THA (△) and PHY (□) on ChE activity. Enzyme preparations were obtained from the striatum of rats. The activity in control animals was 462 ± 53 nmole/mg protein/min. The concentrations of NIK-247, THA and PHY required to inhibit the activity of ChE to half the normal level (IC_{50}) were estimated to be 1.3×10^{-6}, 3.6×10^{-7} and 1.2×10^{-7} M, respectively, from the curves.

least 1 week before the experiments in a room where the temperature (23 ± 0.5°C), humidity (60 ± 5%) and light cycle (12 h illumination with lights turned on at 7:00 a.m.) were controlled to exclude the effects of environmental stress which may influence the activities of enzymes. During this period, the rats received standard food and water.

Chemicals

NIK-247 was a generous donation of Nikken Chemicals Co. Ltd. (Omiya, Japan). PHY and THA were commercially obtained from Sigma (St. Louis, MO). Ethylhomocholine (the internal standard for chromatography) and ACh were purchased from Eicom (Kyoto, Japan) and Sigma, respectively. Reagent-grade chemicals for sample preparation and chromatography were all purchased from a single commercial source (Wako Pure Chemicals, Osaka, Japan) and used without further purification.

In Vitro Experiments

The activities of ChE (8) and ChAT (7) were determined by the procedures reported previously, in which high-performance liquid chromatography with electrochemical detection was employed for the determination of ACh. The animals were killed at between 1300 and 1500 h by decapitation, and the striatum was obtained by the procedure of Glowinski and Iversen (5). The tissue was homogenized in 10 volumes of 50 mM phosphate buffer (pH 7.4) containing 0.5% Triton X-100 using a Polytron homogenizer (Kinematica, Luzern, Switzerland). After centrifugation at 30,000 × g for 30 min, the supernatant was used as a crude enzyme preparation for assays of the enzyme activities. The activities of ChE and ChAT were determined from the consumption and production of ACh in 20 min, respectively.

In Vivo Experiments

The effects of ChE inhibitors on the enzyme activity and on the concentration of ACh in the brain were investigated after intraperitoneal administration of the various drugs. Animals receiving one of the drugs were killed by either decapitation or microwave irradiation, for measurement of the enzyme activities or of the ACh concentrations, respectively, at different times after the administration. For determination of the ChE activities, the same procedures as in the in vitro experiments were applied for making enzyme preparations. The determination of ACh was performed as follows: The striatal sample was first homogenized in 0.1 N HCl. The resultant homogenate was filtered on an ultrafiltration membrane (Type HA; Nihon Millipore, Yonezawa, Japan) by centrifugation, and the filtrant was injected directly into the chromatographic system.

Chromatography

The chromatographic system consisted of a high-pressure pump (Model 510; Waters, Milford, MA), a sample processor (Model 712, Waters), and electrochemical detector with a platinum working electrode (EC-100; Eicom). The analytical column was composed of AC-gel (6.0 i.d. × 150 mm; Eicom) and was connected to an enzyme column (AC-ENZ; Eicom). The detector potential was set at 500 mV vs. the Ag/AgCl reference electrode. The mobile phase consisted of a 0.1-M phosphate buffer (pH 8.0) containing 65 mg/l of tetramethylammonium bromide and 300 mg/l of sodium decansulfonate. The flow rate was set at 1.1 ml/min.

RESULTS

NIK-247 inhibited the activity of ChE in the striatal tissue of rats dose dependently with the effect being initiated at 10^{-7} M (Fig. 1). The effect became almost complete at a concentration of 10^{-4} M. The 50% inhibition concentration (IC_{50}), i.e., the concentration of drug inhibiting the activity to half the non-treated value, was estimated to be 1.3×10^{-6} M. Effects were initiated at concentrations of 10^{-8} M for PHY and THA. With these drugs, dose-dependent inhibitions of the activity of ChE were also observed (Fig. 1). The IC_{50} values were estimated to be 3.6×10^{-7} and 1.2×10^{-7} M for THA and PHY, respectively. This means that the order of in vitro effects on ChE activities was NIK-247 < THA < PHY. Neither NIK-247 nor THA interfered with the activity of ChAT in the striatum of rats at concentrations of less than 10^{-3} M (data not shown).

The in vivo effects of the drugs for inhibition of the ChE activities were examined in striatal samples obtained at different times after intraperitoneal drug administration (Fig. 2A). When the activity levels were determined in animals killed at 30 min after injection of PHY at a dose of 1 mg/kg, the ChE activities were found to be decreased to 25% of those in control animals, in which physiological saline was given. The inhibition reached its maximum effect (20% of the control) at 1 h after the administration. The maximum inhibition of the activities of ChE occurred at 1 h after the administration, being 18, 25 and 30% of control for PHY, THA and NIK-247, respectively. However, the recoveries from the effects were different among the 3 drugs. NIK-247 had the shortest effect on the ChE activity, showing no significant difference when the activity was determined at 2 h after the injection. In the cases of THA and PHY, the ChE activities were significantly inhibited at 2 h after intraperitoneal injection. When the activities were compared at 4 h after the injection, no significant differences were observed for NIK-247 and PHY in comparison with the control. However, the effect of THA on the ChE continued, with a significant decrease in activity still being observed at 4 h after the injection.

The intracerebral concentrations of ACh were increased after the administration of ChE inhibitor (Fig. 2B). The maximum effect was observed at 1 h or 30 min after the administration of NIK-247 or PHY, respectively. However, no significant difference in the concentrations of ACh was noted between 30 min

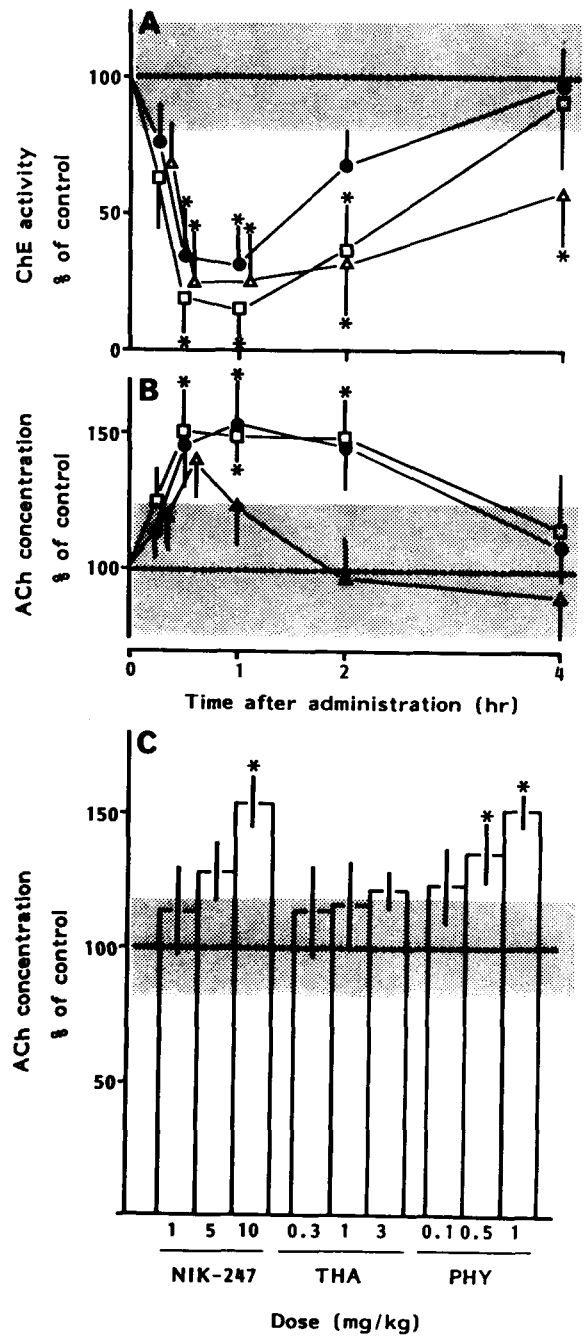

FIG. 2. Effects of NIK-247, THA and PHY on the cholinergic activity in the in vivo experiments. The time courses of the effects of drugs on the ChE activities (A) and concentrations of ACh (B) in the striatum of rats are shown. The animals were injected intraperitoneally with NIK-247 (10 mg/kg; ●), THA (3 mg/kg; △) or PHY (1 mg/kg; □) and then sacrificed at different times after the injection. The dose dependency of the effects of three ChE inhibitors on the concentrations of ACh was also examined in the striatum of rats (C). The drugs were injected at different doses into rats, which were killed at 1 h after the administration.

and 2 h after the administration for these 2 drugs. When the effects were compared at 2 h after drug administration, dose-dependent increases in the concentrations of ACh were observed for NIK-247 and PHY (Fig. 2C). The concentrations recovered at 4 h after the administration of NIK-247 or PHY, although a small elevation was still observed for each drug without any statistically significant change. However, the pattern of change in ACh concentrations differed for THA as compared to both NIK-247 and PHY. THA induced its maximum effect at 30 min after the administration, similarly to PHY, but the concentrations then decreased rapidly after the maximum elevation had been reached. No significant difference was observed even after 2 h, showing almost the same level as in the control.

DISCUSSION

Recent studies have revealed decreases in the concentrations of ACh in the brains of patients with Alzheimer-type dementia. This has generated tremendous interest in the clinical application of ChE inhibitors for such patients. PHY was the first drug to be used with some benefit, and THA was then introduced for this purpose (10). However, the clinical application of THA is limited because of the toxicity of this drug (9). Recent reports have suggested that THA possesses a hepatotoxicity (3) and a possible neurotoxicity interacting with glutamate receptors (15). Efforts have thus been directed towards the development of new ChE inhibitors with a greater potency and/or less toxicity. NIK-247 is a newly introduced analogue of THA and has been reported to attenuate some of the behavioral abnormalities in shuttle-box learning observed in rats with experimentally induced amnesia (11,13). However, no neurochemical studies have yet been carried out on the efficacy of NIK-247 for the inhibition of ChE activities.

NIK-247 was less effective in its inhibition of ChE activity than the other drugs, PHY and THA, when their effects were compared in in vitro experiments. The potency of NIK-247 was about 1/10 and 1/3 that of PHY and THA, respectively. Thus, in the in vivo experiments, we administered 1, 3 and 10 mg/kg of PHY, THA and NIK-247, respectively, in order to compare the duration of the effects of such ChE inhibitors on enzyme activity and transmitter in rats. Maximum inhibition of the enzyme was noted at 30 min to 1 h after intraperitoneal administration of the drugs. In the case of PHY and NIK-247, the activities of ChE almost recovered within 4 h, but a decreased activity (50% of control) still remained at 4 h with THA. This means that, although the effect of THA is long lasting, NIK-247 exerts a transient effect on ChE activity, being similar to PHY, the prototype of this drug class. Such a difference in the duration of the in vivo effect of ChE inhibitors has been reported previously (6). The intrastriatal concentrations of ACh were changed in correlation to the ChE activities for PHY and NIK-247, with maximum effects being observed at between 30 min and 1 h after the administration, respectively. With these drugs, the concentration recovered at 4 h after the administration, which was similar to the time course of the effect of drugs on ChE activity. It might be concluded that these results indicate that the concentration of ACh was simply regulated by the activity of its synthesizing enzyme. However, the concentration recovered to a normal level within 2 h in animals given the long-lasting THA. At that time, the activity of ChE was still decreased. These findings suggest that the concentrations of ACh were not simply regulated by the activity of ChE, the degrading enzyme of this transmitter. Similar data for the ACh level have been reported previously (6). One possible explanation is that some influence of THA on the activity of ACh-synthesizing enzyme, ChAT, may be included in its effect. In the present study, however, no significant changes were observed in the activity of ChAT for both NIK-247 and THA. It has been demonstrated that THA influences the uptake mechanism of choline, the precursor of ACh

(2). It is possible, therefore, that the transient effect of THA on the concentration of the transmitter might be related to inhibition of the precursor uptake mechanism.

The present drug group, comprising ChE inhibitors, has been applied clinically for the treatment of peripheral and central cholinergic deficits, in which the concentrations of ACh are decreased. The effect of NIK-247 resembled that of PHY much more than that of THA. The results reported here suggest that NIK-247 increased the intracerebral concentration of ACh and might be effective for the treatment of such deficits, although evaluation of the detailed toxicology is needed. Thus NIK-247 should be considered for possible clinical use instead of THA.

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