

***In vitro* Anticholinesterase Activity of Various Alkaloids**

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Z. Naturforsch. **62c**, 684–688 (2007); received March 15/May 2, 2007

In the current study, a number of alkaloids including retamine, cytisine, and sparteine (quinolizidine-type), yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin (imidazolidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type) were tested *in vitro* for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) at 1 mg/ml concentration by the Ellman method using an ELISA microplate reader. Among the alkaloids tested, only capsaicin exerted a remarkable inhibitory effect towards both AChE and BChE [(62.7 ± 0.79)% and (75.3 ± 0.98)%, respectively]. While the rest of the alkaloids did not show any significant inhibition against AChE, three of the alkaloids, namely retamine, sparteine, and yohimbine, exerted a noteworthy anti-BChE effect as compared to galanthamine, the reference drug.

Key words: Alkaloid, Acetylcholinesterase, Butyrylcholinesterase

Introduction

Although there is currently no cure for Alzheimer's disease (AD), new treatments are on the horizon as a result of accelerating insight into the biology of the disease. Acetylcholinesterase (AChE) inhibition has been thought to be quite important for the reduction of activity of the cholinergic neurons detected in the brains of AD patients (Schmitz *et al.*, 2004). AChE inhibitors diminish the rate at which acetylcholine (ACh) is broken down and hence increase the concentration of ACh in the brain (Shen, 2004; Samanta *et al.*, 2006). The commercially available AChE inhibitors such as tacrine, donepezil, rivastigmine, or galanthamine may help to prevent, for a limited time, some symptoms from becoming worse for some people in the early and middle stages of the disease (Luckmann, 2006; Haviv *et al.*, 2007).

In our ongoing work on identifying new natural inhibitors of AChE and BChE for the treatment of AD, we herein screened thirteen alkaloids with a variety of chemical skeletons, namely retamine, cytisine, and sparteine (quinolizidine-type), yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine

(tropolone-type), allantoin (imidazolidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type), by the spectrophotometric method of Ellman at 1 mg/ml concentration.

Material and Methods

Tested alkaloids

The thirteen alkaloids used in this study, namely sparteine (Aldrich-173738), yohimbine (Sigma-Y3125), vincamine (Sigma-V2127), scopolamine (Sigma-S0929), atropine (Sigma-A0132), colchicine (Sigma-C9754), allantoin (Sigma-A7878), trigonelline (Sigma-5509), octopamine (Sigma-O0250), synephrine (Sigma-S0752), and capsaicin (Sigma-V9130), were purchased from the respective manufacturers. Retamine and cytisine were isolated from *Genista aucheri* Boiss. (Fabaceae) as described previously (Tosun, 1986).

Determination of AChE and BChE inhibitory activities

AChE and BChE inhibitory activities were measured by slightly modifying the spectrophoto-

metric method developed by Ellman *et al.* (1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7; Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8; Sigma) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were employed as substrates of the reaction. 5,5'-Dithio-bis(2-nitrobenzoic)acid (DTNB; Sigma) was used for the measurement of the cholinesterase activity. All other reagents and conditions were the same as described in previous publications (Atta-ur-Rahman *et al.*, 2000; Orhan *et al.*, 2004, 2007). Briefly, in this method, 140 μ l of 0.1 mM sodium phosphate buffer (pH 8.0), 20 μ l of DTNB, 20 μ l of test solution and 20 μ l of AChE/BChE solution were added by a multichannel automatic pipette (Gilson pipetman, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 μ l of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (SpectraMax Plus-384, Molecular Devices, Union City, CA, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of the rates of reaction of the compounds relative to a blank sample (ethanol in phosphate buffer, pH 8) using the formula $(E-S)/E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test compound. The experiments were performed in triplicate in each case. Galanthamine, the anticholinesterase alkaloid-type of drug isolated from the bulbs of snowdrop (*Galanthus* sp.), was purchased from Sigma and was used as reference.

Statistical analysis

Data obtained from *in vitro* experiments are expressed as mean standard error (\pm SEM). Statistical differences between the treatments and the control were evaluated by ANOVA test. $P < 0.05$ was considered to be significant [$* P < 0.05$; $** P < 0.01$; $*** P < 0.001$].

Results and Discussion

Acetylcholinesterase (AChE) inhibitors are an important class of medicinal agents useful for the

treatment of AD, glaucoma, myasthenia gravis, and for the recovery of neuromuscular block in surgery. Therefore, in this study, *in vitro* AChE and BChE inhibitory activities of thirteen alkaloids (Fig. 1), *i. e.* retamine, cytisine and sparteine (quinolizidine-type), yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin (imidazolizidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type), were tested by the spectrophotometric method of Ellman at 1 mg/ml concentration. As seen in Table I, cytisine, colchicine, allantoin, trigonelline, and synephrine were completely inactive against both enzymes, while only capsaicin was significantly active on AChE and BChE. The anti-AChE effect of sparteine was moderate as it was below 50%. Capsaicin was found to display the highest inhibitory effect against BChE, which was the closest to galanthamine, followed by sparteine, yohimbine, and retamine.

Nitrogen-containing compounds, especially alkaloids from herbal sources such as galanthamine and huperzine A as the most popular ones, have been so far proven to exert a remarkable anticholinesterase activity (Liu *et al.*, 1986; Heinrich and Teoh, 2004). Although huperzine A, having a quinoline skeleton, as well as galanthamine with an isoquinoline skeleton, a potent competitive and reversible anticholinesterase alkaloid occurring

Table I. AChE and BChE inhibitory activities of thirteen alkaloids^a.

Alkaloids	Inhibition (%) at 1 mg/ml	
	AChE	BChE
Retamine	15.0 \pm 1.08***	66.3 \pm 0.88***
Cytisine	– ^b	–
Sparteine	41.6 \pm 1.62	73.9 \pm 1.03***
Yohimbine	–	73.4 \pm 1.25***
Vincamine	–	28.5 \pm 2.01***
Scopolamine	–	23.8 \pm 1.98***
Atropine	–	44.4 \pm 0.57***
Colchicine	–	–
Allantoin	–	–
Trigonelline	–	–
Octopamine	–	36.1 \pm 1.57***
Synephrine	–	–
Capsaicin	62.7 \pm 0.79***	75.3 \pm 0.98***
Galanthamine	99.8 \pm 0.31	80.3 \pm 1.14

^a Values are expressed as mean \pm SEM ($n = 3$).

^b No inhibition.

$P > 0.05$; $* P < 0.05$; $** P < 0.01$; $*** P < 0.001$.

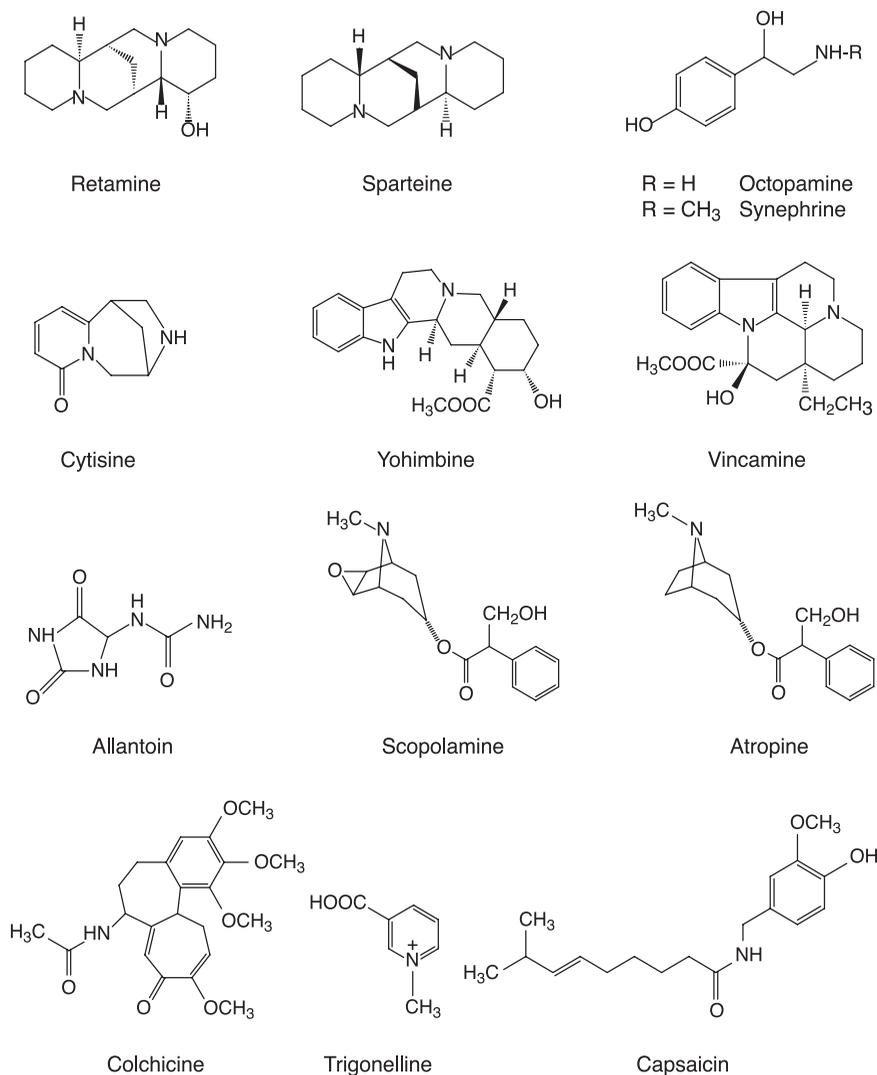


Fig. 1. Chemical structures of the tested alkaloids.

widely in Amaryllidaceae, have been shown to be the most promising anticholinesterase drugs belonging to the quinolizidine-type alkaloids; retamine, cytisine and sparteine studied herein were inactive against AChE. However, retamine and sparteine showed an appreciable inhibition towards BChE. At this instant, it might be suggested to have a look into structure-activity relationship of huperzine A. According to Kozikowski *et al.*'s study (1996), an axial methyl group attached to the C-10 position of huperzine A increased the potency for AChE inhibition 8-fold, while the corresponding equatorial isomer was about 1.5-fold less

active than huperzine A. The introduction of substituents larger than methyl resulted in a drop in activity.

On the other hand, Maizel *et al.* (1978) reported that the rate of AChE hydrolysis decreased in the order of ephedrine, salsoline, and cytisine with the volumetric increase of the cationic group, while the decrease was almost 10-fold for BChE. In another study, a number of analogues of acetyl- β -methylcholine, containing residues of the alkaloids anabasine and cytisine as cyclic ammonium groups, were synthesized and proved to be reversible inhibitors of AChE and BChE (Dobren'kov *et*

al., 1988). However, cytisine was entirely ineffective in our study, whereas retamine and sparteine had a noteworthy inhibition on BChE, which might result from their bis-quinolizidine skeletons, while cytisine has only quinolizidine ring in its molecular structure. Similarly, in our screening, vincamine, an indol alkaloid attached with a quinolizidine ring, was also not active against these enzymes. However, yohimbine, another indoloquinolizidine alkaloid rather similar to vincamine, exhibited a good anti-BChE effect, which might be due to a slight difference on the location of ring E (Fig. 1). In fact, yohimbine and its isomers were reported to be weak inhibitors of rat-brain AChE and weak antagonists at muscarinic cholinergic receptors (Lambert *et al.*, 1978), but there has been no report on its effect on BChE.

Interestingly, *Vinca minor* L. has been traditionally used as tea for elderly people suffering from dementia (Calvo, 2003). Vincamine as a major component of *V. minor* L. has been shown to be ineffective in our assay. Nevertheless, its positive effect on dementia patients could be resulted from its vasodilatory effect by improving cerebral circulation. On the other hand, vinpocetine, a synthetic ethyl ester derivative of vincamine, was found to have cholinergic activity and seems to be able to protect neurons against oxidative stress (Thal *et al.*, 1989). It was also shown in a few short-term studies that vinpocetine can moderately improve cognitive functions in AD patients (Szatmari and Whitehouse, 2003).

On the other hand, scopolamine is an alkaloid well-known for its amnesia-inducing effect, and atropine, which is used as cycloplegic and midriatic, is an anticholinergic agent that is a potent parasympatholytic, inhibiting actions of acetylcholine at postganglionic parasympathetic neuroeffector sites (Rush, 1988). It is a competitive antagonist of

acetylcholine at smooth and cardiac muscles and various glandular cells. By blocking the action of acetylcholine at muscarinic receptors, atropine also serves as an antidote for poisoning by organophosphate insecticides and nerve gases. Therefore, futility of scopolamine and atropine as being anticholinergic agents of AChE inhibition was not surprising.

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is the active component of chili peppers that are plants belonging to the genus *Capsicum*. In 1981, Papka *et al.* figured out that AChE activity in nerve fibers of guinea pig was depleted by capsaicin as well as diminution of substance P immunoreactivity. Definitely, in an analogous study by Gamse *et al.* (1982), capsaicin (10 mg/ml) led to a complete block of the axoplasmic transport of immunoreactive substance P and somatostatin in rat sciatic nerves without affecting the transport of noradrenaline or acetylcholinesterase. In a later study (Duckles and Levitt, 1984), it was revealed that activities of choline acetyltransferase and acetylcholinesterase were also unchanged after capsaicin treatment. However, it is important to mention that we found that capsaicin displayed a noteworthy *in vitro* inhibition of AChE and BChE.

In conclusion, this paper's intent was to focus on *in vitro* AChE and BChE inhibitory effects of thirteen alkaloids of various chemical classes. Although retamine, sparteine, yohimbine, and capsaicin were shown to be highly active against BChE, only capsaicin was a prominent inhibitor of AChE at 1 mg/ml. In order to establish the structure-activity relationship for the active compounds studied, QSAR studies could be suggested to be carried out. To the best of our knowledge, this is the first report on the anticholinesterase activity of the above-mentioned alkaloids except for cytisine and yohimbine.

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