魚類麻酔剤2-アミノ-4-フェニールチアゾールのマゴイにおける中枢作用性の生理化学的解析

誌名	日本水産學會誌
ISSN	00215392
著者	関沢, 泰治 ほか3名,
巻/号	40巻4号
掲載ページ	p. 351-361
発行年月	1974年4月

Bulletin of the Japanese Society of Scientific Fisheries 40(4) 351-361 (1974)

Action Mechanism of 2-Amino-4-phenylthiazole, a Piscine Anesthetic, Upon the Central Nervous System of Carp*1

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(Received December 17, 1973)

The physio-chemical characteristics of a sedative, 2-amino-4-phenylthiazole, as to its anesthetic properties upon carp (*Cyprinus carpio*) were surveyed. (1) EEG activation by acetylcholine chloride administered into the cerebellum was observed under concomitant debilitation of EEG from the cerebellum by anesthetization. EEG activation was also observed by serotonin-creatinine sulfate or dopamine hydrochloride, but not by noradrenaline hydrochloride. (2) This compound gave neither extreme activitation nor inhibition upon the monoamine oxidase (EC 1.4.3.4) or acetylcholine hydrolase (EC 3.1.1.7) activity. (3) The depletion of acetylcholine in the nerve ending particles was observed under anesthetization. The depletion of serotonin or dopamine was not observed. It might be concluded that the depletion of acetylcholine resulted in the inhibition of choline o-acetyltransferase (EC 2.3.1.6) activity and the abatement of acetylcholine binding capacity of the synaptic vesicles in the presence of this compound. (4) The brain cAMP was observed to be increased by the anesthetization. Although the implications as for the augmentation of brain cAMP was not yet fully elucidated, it was concluded that the augmentation resulted from inhibition of cAMP phosphodiesterase (EC 3.1.4.1) activity in the presence of this compound.

As described in the previous reports, 2-amino-4-phenylthiazole had CNS acting property as for the main electrophysiological characteristic,[1] and the major distribution of the tritiated compound in carp body was found in brain reinforcing its encephalotropic characteristics.[2] From these experimental evidences, the research has been focused upon its drug action mechanism in carp CNS in the sense of physiological chemistry. The purpose of the present study is to elucidate whether chemical transmitters activate the devilitated EEG under the anesthetization with this compound relating to the site of its action along neuron system, whether this compound has influence on the storage of chemical transmitters in NEPs and further this compound has influence on the brain enzymic activities as for monoamine oxidase, acetylcholine hydrolase, choline o-acetyltransferase and cAMP phosphodiesterase relating to its action in enzymic level.

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*1 The outline of this study was given at The International Meeting of The International Society for Neurochemistry: *Abstract*, p. 484, No. 641, Tokyo (1973).

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Abbreviation: CNS=central nervous system. EEG=electroencephalogram. NEPs=nerve ending particles. cAMP=adenosine 3′: 5′-cyclic monophosphate. FAD=flavine adenine dinucleotid dinucleotide. TLC=thin layer chromatography.

Materials and Methods

Determination of EEG activation The determination of EEG from stratum basalis of carp cerebellum and of the debilitation of the EEG by the anesthetization with 2-amino-4-phenylthiazole were previously described.[1] Zero point five μ mole of chemical transmitter including serotonin-creatinine sulfate, dopamin hydrochloride, acetylcholine chloride or noradrenaline hydrochloride was respectively given iontophoretically with glass capillary or mechanically through fine polyvinyl tubing connected with glass capillary near at the fine concentric measuring electrode.

Determination of monoamine oxidase and acetylcholine hydrolase activity Carp brain homogenate suspension was prepared using 2.3 g of carp whole brains which were homogenized in Teflon homogenizer and the resulted homogenate was further suspended in 10 m*l* of 100 mM phosphate buffer solution at pH 7.4. All operation was done at 0–5°C. For assay of the monoamine oxidase activity, the brain homogenate suspension was pre-incubated with 1mg FAD per 10 m*l* of the suspension for 2 hr at 25°C prior to use. The reaction mixture for assay of the monoamine oxidase activity consisted of 8 m*l* of 100 mM phosphate buffer solution at pH 7.4 containing 1 mM NaCN to inhibit high endogenous respiration of brain tissue, 2 mM of each of serotonin-creatinine sulfate, dopamine hydrochloride or noradrenaline hydrochloride plus 2 m*l* of the brain homogenate suspension in the presence or absence of 2-amino-4-phenylthiazole hydrobromide at 2.5 mM. The consumption of dissolved oxygen was measured polarographically at 37°C as previously described.[1] The oxidation of the respective biogenic amine was proceeded along sigmoid curve in each recording polarographical run. The maximum velocity at the deflection point of the sigmoid curve was taken as the vleocity of the oxidation, when the deflection point was clearly obtained. Reading was taken usually within or at 60 min incubation in 10 m*l* closed type vessel devised with platinum and calomel electrode under magnetic stirring.

The determination of acetylcholine hydrolase activity of the brain homogenate suspension was done according to Voss and Sachese.[3] The reaction mixture consisted of 3 m*l* of 50 mM phosphate buffer solution at pH 7.4 containing 10 mg per cent of sodium 5′,5′-dithio-bis-(2-nitrobenzoate), 0.1 m*l* of 156 mM of acetylthiocholine iodide aqueous solution plus 1.9 m*l* of the brain homogenate suspension diluted with 50 mM phosphate buffer solution at pH 7.4 in the presence or absence of 2-amino-4-phenylthiazole hydrobromide at 2.5 mM. Prior to electrophotometric reading, the respective reaction mixture was filtered through Millipore filter: HAWG #01300, HA 0.45 μ (Japan Millipore, Ltd., Tokyo). Reading was taken electrophotometrically at 405 mμ after 5 min incubation at 37°C.

Determination of chemical transmitters contents of nerve ending particles The fraction-

ation of NEPs from carp brain homogenate was done according to the similar procedure described by WITTAKER, MICHAELSON and KIRKLAND.[4] The all operation was done at 0–5°C. The diagram of the fractionation was shown in Fig. 1.

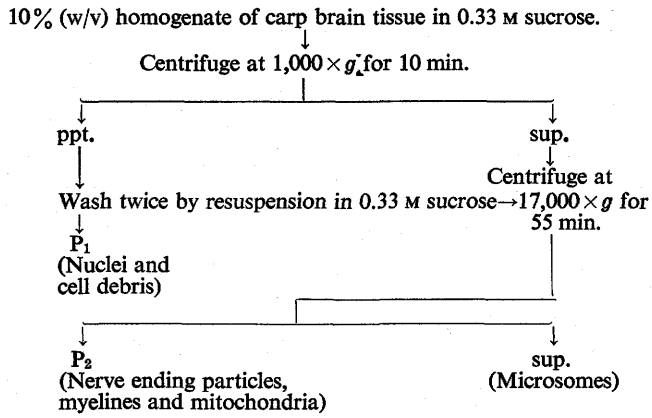

Fig. 1. Schematic diagram for fractionation procedure of nerve ending particles from carp brain.

Fraction p_2 was used for further studies as crude NEPs fraction. As for the acetylcholine contents, each run was started from at least 6 carp brains weighing 3 to 3.6 g in total. The crude NEPs fraction was washed once by 0.33 M sucrose solution employing Thermo mixer and subjected to centrifuge under 17,000×g for 55 min at 0–5°C. To the precipitated NEPs fraction weighing 1 g, 1 m*l* of distilled water was added and a half part of the suspension was again subjected to centrifuge for 55 min. The resulted supernatant was used for the determination of acetylcholine under unstable binding state. Another half part in small tube was freezed at −18°C over night. After thawing, it was heated in boiling water bath for 10 min. After cooling, the resulted supernatant under centrifuge was used for total acetylcholine assay. Acethylcholine was determined biologically using excised rectum of carp in TYRODE's solution or excised heart of clam in sea water at 22°C. The sensitive site in carp rectum was found approximately at 5 cm from the anus of 2 years old carp. The presence of 2-amino-4-phenylthiazole hydrobromide at 0.5 mM or cAMP at 1 μM did not practically interfere its quantitativity. The application of force-displacement transducer Nihon Koden Model SB–1T improved sensitivity. The electronic amplifier used was Sanei Type 1206. The linear relationships between acetylcholine and the amplitude of contraction were given between 4 and 0.2 μM at 40 mm Hg position and between 0.4 and 0.04 μM at 10 mm Hg position when semilogarithmic section paper was used for plotting. The assay with heart of clam in sea water was semiquantitative method and the addition of more than 0.01 μM of acetylcholine to the good preparation caused retardation of the beating pace.

As for other chemical transmitters contents in NEPs, the experimental run was

started from 20 carp brains. The experimental procedure was similar as described by BOGDANSKI, PLETSCHER, BRODIE and UNDENFRIEND.[5] NEPs suspension was once directly extracted with 3 fold volume of n-butanol at pH 1.0 adding diluted hydrochloric acid, and after the separation of n-butanol layer, the aqueous layer containing NEPs suspension was adjusted to pH 10.0 adding diluted sodium hydroxide. The alkaline aqueous suspension was extracted with 2 fold volume of n-butanol 3 times and the pooled n-butanol under alkaline extraction was concentrated under vacuum to almost dryness. To the concentrate, 0.1 m*l* of dried methanol was added, and the resulted solution was applied on Merck TLC plate. Serotonin and dopamine were determined by chromatogenesis under light still-standing for 2 days on TLC plate using Shimazu CS-900 dual wave length sweep type scanning machine. Three hundred and fifty and 720 mμ lights were used for sample and reference sites respectively. n-butanol-acetic acid-water 5-1-2 (v/v) was used for developing solvent. Rf for serotonin=ca. 0.60 and for dopamine=ca. 0.44. The limit of determination as for both serotonin and dopamine on the developed TLC plate was 0.25 μg. To compare chemical transmitters contents in NEPs between normal and anesthetized carp brain, the anesthetization was done in a bath containing 25 ppm of 2-amino-4-phenylthiazole hydrobromide at 22°C for 4 hr.

Determination of choline o-acetyltransferase and cAMP phosphodiesterase activity The preliminary survey on the distribution of the choline o-acetyltransferase activity in carp NEPs indicated that the major activity was found in crude synaptic vesicles preparation after the hypotonic treatment, although fractions derived from NEPs were not eserinized in the present study and it was yet uncertain whether the choline o-acetyltransferase normally bound in synaptic vesicles fraction in carp brain. In the present study, the vesicles collected by high speed centrifuge at 100,000×g after the hypotonic treatment of about 10 g carp NEPs fraction per 50 m*l* of distilled water, were used for the enzyme source. On the other hand, to confirm the inhibitory action of 2-amino-4-phenylthiazole on choline o-acetyltransferase activity, the crude enzyme from mammalian source was prepared from porcine forebrain applying the extraction and purification procedure described by BERMAN, WILSON and NACHMANSON[6] and further purification was done according to GLOVER and POTTER.[7] The reaction mixture usually consisted of 10 mM of choline chloride, 2.5 mM of acetyl-CoA, 5 mM of magnesium chloride, 1 mM diisopropylfluorophosphate and 1.4 m*l* of the crude synaptic vesicles suspension or 0.1 m*l* of crude porcine brain choline o-acetyltransferase with or without 1.1 mM of 2-amino-4-phenylthiazole acid salt. Total volume of the reaction mixture for the carp brain enzyme was 2.0 m*l* and the total volume for the porcine brain enzyme was 1.0 m*l* in 100 mM phosphate buffer solution at pH 7.4. The both synthetic reactions were operated at 37°C for 60 min. The acetylcholine found in the reaction mixture was determined by the biological assay above described after stopping of the reaction by heating in boiling water bath. The

endogenous acetylcholine was not detected in the respective enzyme source.

The semiquantitative determination of cAMP was done biologically using myxamoeba (*Dictyostelium discoideum* NC-4) according to a similar procedure described by KONIJN.[8] The chemotactic responce (per cent) was obtained averaging the 50 chemotactic reactions for one sample on the agar plate. 2-amino-4-phenylthiazole at 1 mM did not interfere the chemotactic response of myxamoeba by cAMP.

To compare cAMP contents between normal and anesthetized carp brain, the anesthetization was done in a bath containing 25 ppm of 2-amino-4-phenylthiazole hydrobromide at 22°C for 4 hr. The brains were taken out rapidly within 1 min at room temperature after decapitation. The brains were immediately cooled at 0°C and homogenized by Teflon homogenizer in 100 mM phosphate buffer solution at pH 7.4 under 10 w/v% at 0–5°C. After centrifugation at 17,000×g at 5°C, the supernatants which contained the extracted cAMP were freez-dried to be used for assay sample.

To determine the influence of this compound on cAMP phosphodiesterase activity, the reaction mixture consisted of 1 μM of cAMP, 10 μl of bovine heart cAMP phosphodiesterase (Böhringer Mannheim, West Germany) and 5 μM of magnesium chloride with or without 0.1 mM of 2-amino-4-phenylthiazole acid salt. The reaction was operated at 37°C for 30 min.

Results

EEG activations by biogenic amines under the anesthetization with 2-amino-4-phenylthiazole in carp To find a clue prior to physio-chemical study, electrophysio-logical survey on the site of drug action along neuron system is quite important. Since the electrophysiological studies had been reported previously,[1] the EEG activation in carp cerebellum under the anesthetization was noted. The results regarding the EEG activations were summarized in Fig. 2. Chart 1 in the figure showed the EEG from the stratum basalis of carp cerebellum in normal run usually giving 16–18 Hz brain wave. Chart 2 showed EEG at 10 min in the course of anesthetization by bathing of this compound at 40 ppm and 22°C indicating the debilitation of normal EEG. Chart 3, 4 or 5 showed respectively a mimic brain wave evoked by the administration of each 0.5 μ mole of serotonin-creatinine sulfate, dopamine hydrochloride and acetylcholine chloride. The administration of noradrenaline hydrochloride seemed likely not to evoke an activated brain wave. The duration time of EEG activation was generally a few to several seconds. The chemical species of acid salt including hydrobromide, hydrochloride or methanesulfonate gave the similar results.

The results obtained seemed to suggest that post nerve fibers including post synaptic membrane are still active under the anesthetization. This experimental fact, with the previously described evidence in which this compound lacked the direct inhibitory action

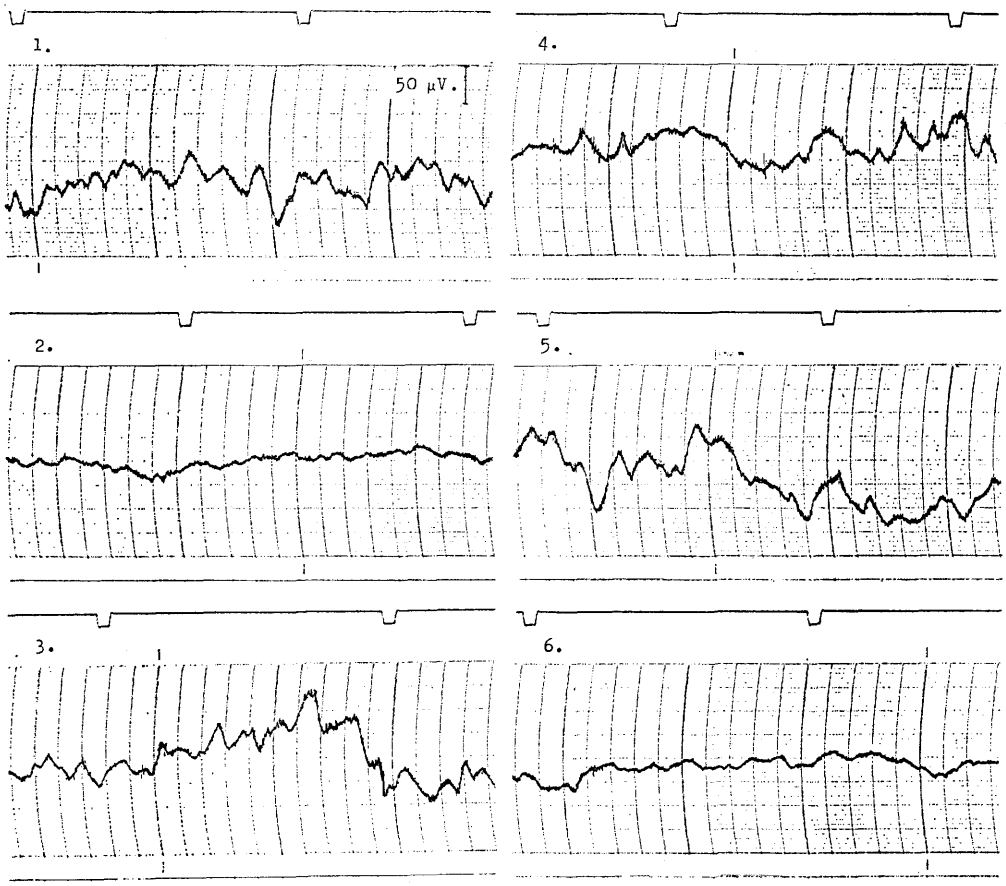

Fig. 2. EEG activations by biogenic amines under the anesthetization with 2-amino-4-phenylthiazole in carp.
1. Normal EEG from cerebellum. 2. Debilitation of EEG at 10 min in the course of the anesthetization. 3. Administration of serotonin-creatinine sulfate. 4. Dopamine hydrochloride. 5. Acetylcholine hydrochloride. 6. Noradrenaline hydrochloride. Time mark=1 sec. Voltage scale=50 μV. Further explanations were described in the text.

upon the conduction along pre-nerve fiber, seemes to imply that the site of action of this compound in CNS should be the nerve ending portions of pre-nerve fibers where synaptic vesicles store chemical transmitters.

Influence of 2-amino-4-phenylthiazole upon monoamine oxidase and acetylcholine hydrolase activity of carp brain homogenate Relating to sedative to anesthetic property of 2-amino-4-phenylthiazole, surveys were done whether this compound activated extremely the activity of monoamine oxidase or acetylcholine hydrolase in carp brain. The result regarding the influence of this compound on monoamine oxidase of carp brain homogenate was shown in Table 1. Although 2.5 times amounts of this compound usually found in carp brain during the anesthetization was added,[2] neither extremely excitatory nor inhibitory action was observed as shown in the table.

Table 1. Influence of 2-amino-4-phenylthiazole upon the monoamine oxidase of carp brain homogenate

Substrate	Additive	Biogenic amine oxidized (μg/ml/hour)	Activation (%)
serotonin	none	33.8	
serotonin	2-amino-4-phenylthiazole*	30.0	−11
dopamine	none	6.0	
dopamine	2-amino-4-phenylthiazole*	6.0	0
noradrenaline	none	5.2	
noradrenaline	2-amino-4-phenylthiazole*	5.2	0

* The concentration in reaction mixture=2.5 mM.

Table 2. Influence of 2-amino-4-phenylthiazole upon the acetylcholine hydrolase of carp brain homogenate

Substrate	Additive	Acetylthiocholine hydrolized (μg/ml/hour)	Activation (%)
acetylthiocholine	none	528	
acethylthiocholine	2-amino-4-phenylthiazole*	540	+2

* The concentration in reaction mixture=2.5 mM.

Table 3. Chemical transmitters contents of nerve ending particles fractionated from normal or anesthetized carp brains

Transmitter substances	Normal brain		Anesthetized brain	
	Transmitter μg/g wet NEPs			
acetylcholine eq.	3,	5	0.1,	0.2
serotonin		0.6		0.5
dopamine		2.3		1.5
acetylcholine eq. bound (%) as stable state.	28,	35	0	0

Table 4. Chemotactic response of myxamoeba (*Dictyostelium discoideum* NC-4) induced by normal or anesthetized carp brain extracts

	Standard cAMP run 10^{-9} M	10^{-8} M	Normal brain*	Anesthetized brain*
Chemotactic responce (%)	12	67	9	58

* Both carp brain extracts were freeze-dried and then dissolved in a half volume of excised brains.

The result regarding the influence of this compound on acetylcholine hydrolase of carp brain homogenate was shown in Table 2. Although 2.5 times amounts of this compound usually found in carp brain during the anesthetization was also added, neither extremely excitatory nor inhibitory action was observed as shown in the table. Regarding both enzymic reactions including monoamine oxidase and acetylcholine hydrolase, other chemical species of acid salt, such as hydrobromide, hydrochloride or methanesulfonate also gave the similar results.

Table 5. Influence of 2-amino-4-phenylthiazole upon the choline o-acetyltransferase of carp or porcine brain

	Carp brain			Porcine brain		
Reaction mixture	I	II	III	I	II	III
choline chloride	1.2×10^{-6} M	1.2×10^{-6} M	—	10^{-2} M	10^{-2} M	—
acetyl-CoA	1.2×10^{-6} M	1.2×10^{-6} M	—	2.5×10^{-3} M	2.5×10^{-3} M	—
$MgCl_2\cdot6H_2O$	5.0×10^{-3} M	5.0×10^{-3} M	5.0×10^{-3} M	5.0×10^{-3} M	5.0×10^{-3} M	5.0×10^{-3} M
2-amino-4-phenylthiazole	—	10^{-3} M	—	—	1.1×10^{-3} M	—
diisopropyl-fluorophosphate	—	—	—	10^{-3} M	10^{-3} M	10^{-3} M
crude synaptic vesicles suspension (carp brain)	1.4 m*l*	1.4 m*l*	1.4 m*l*	—	—	—
crude choline o-acetyltransferase (porcine brain)	—	—	—	0.1 m*l*	0.1 m*l*	0.1 m*l*
acetylcholine equ. found	28 ng/m*l*	2 ng/m*l* >	2 ng/m*l* >	68 ng/m*l*	1 ng/m*l*	trace
inhibition (%)		ca.93% <			98%	

Table 6. Influence of 2-amino-4-phenylthiazole upon the cAMP phosphodiesterase of bovine heart

Reaction mixture	I	II	III
cAMP	10^{-6} M	10^{-6} M	10^{-6} M
$MgCl_2\cdot6H_2O$	5×10^{-3} M	5×10^{-3} M	—
2-amino-4-phenylthiazole	—	10^{-4} M	—
cAMP phosphodiesterase	10 μl	10 μl	—
Myxamoeba chemotactic responce (%) at 100×dilution			
initial	39.3	35.0	41.5
incubation for 30 min at 37°C	8.0	56.4	—

Table 7. Influence of 2-amino-4-phenylthiazole upon the binding of acetylcholine in synaptic vesicles fractionated from porcine brain

Reaction mixture	I	II	III	IV
acetylcholine chloride	—	10^{-2} M	10^{-2} M	—
2-amino-4-phenylthiazole	—	—	0.9×10^{-3} M	0.9×10^{-3}M
H_2O	80 μl	40 μl	—	40 μl
synaptic vesicles fraction	2.5 m*l*	2.5 m*l*	2.5 m*l*	2.5 m*l*
acetylcholine eq. released (ng/m*l*)	0	34.4	16.3	0
inhibition (%)			53%	

These experimental evidences described herein implied that the sedative to anesthetic property of 2-amino-4-phenylthiazole did not involve the monoamine oxidase or acetylcholine hydrolase activity in carp brain.

Influence of 2-amino-4-phenylthiazole upon the contents of chemical transmitters in nerve ending particles obtained from carp brain and the contents of cAMP in carp brain during the anesthetization The contents of chemical transmitters in NEPs fractionated

from carp brain homogenate under normal or anesthetized condition were investigated. The results were summarized in Table 3.

The marked depletion of acetylcholine in the nerve ending particles was noted with the contrastable results regarding other chemical transmitters remained to be practically unchanged after the anesthetization at 25 ppm and 22°C for 4 hr.

On the other hand, when cAMP contents were compared between normal or the anesthetized brain, the augmentation of cAMP was noted on the anesthetized run as shown in Table 4.

Hence the myxamoeba used was highly specific to cAMP for its aggregation, the augmentation of cAMP in the anesthetized run was confirmed.

Influence of 2-amino-4-phenylthiazole upon choline o-acetyltransferase and cAMP phosphodiesterase activity The crude synaptic vesicles suspension in 100 mM phosphate buffer solution, which was derived from carp NEPs fraction, was used for the enzyme source of choline o-acetyltransferase. The other source of the enzyme partially purified from porcine forebrain was used for the reference run. The incubations were done at 37°C for 60 min. The acetylcholine formed was assayed biologically after heating in boiling water bath. The results were summarized in Table 5. The acid salt of 2-amino-4-phenylthiazole used was metahnesulfonate, other salt such as hydrobromide gave the similar results.

From the experimental results, it was indicated that 2-amino-4-phenylthiazole inhibited the activity of choline o-acetyltransferase derived from both carp and porcine brain. These evidences revealed that the specific depletion of acetylcholine described above might be mainly caused by the inhibition of choline o-acetyltransferase activity by this compound. Recently HEBB, STEPHENS and SMITH pointed out that the optimum temperature for the enzymic activity of choline o-acetyltransferase derived from poikilotherm brain strictly depended on the rearing temperature and that the preparation of this enzyme from the brain of poikilotherm such as gold fish involved some difficulties. The lower productivity of acetylcholine in the above experimental run as for carp choline o-acetyltransferase was thus explained later by the above evidences, although the concentrations of substrates in this run were taken as in a physiological concentration.

On the other hand, the influence of 2-amino-4-phenylthiazole (hydrobromide) upon bovine heart cAMP phosphodiesterase activity was investigated. The result was shown in Table 6.

It was indicated that the augmentation of cAMP in carp brain should be mainly caused by the inhibition of cAMP phosphodiesterase activity by this compound.

Influence of 2-amino-4-phenylthiazole upon the binding of acetylcholine in synaptic vesicles fraction When the washed crude synaptic vesicles fraction was incubated with acetylcholine at 37°C for 60 min, the vesicles bound exogenous acetylcholine. In the

step of the binding, the presence of this compound (hydrobromide) inhibited the binding of acetylcholine as shown in Table 7.

This evidence suggested that the depletion of acetylcholine in NEPs described above was possibly also caused by the abatement of the binding capacity of acetylcholine in the synaptic vesicles with this compound.

Discussion

From the electrophysiological evidences regarding the EEG activation described in this study, the site of action of 2-amino-4-phenylthiazole along neuron system in carp CNS seemed to be pre-nerve fiber endings. The fraction containing NEPs was separated from carp brain homogenate, and the specific depletion of acetylcholine in the NEPs was observed during the course of the anesthetization with this compound. In fact, the contents of other excitatory chemical transmitters, serotonin and dopamine, were practically unchanged during the course of the anesthetization. Two explanations for the depletion of acetylcholine in carp NEPs became to be available in the extent of this study, one was that 2-amino-4-phenylthiazole inhibited the activity of acetylcholine synthesizing enzyme, choline o-acetyltransferase, at the concentration required for the anesthetization, and another was that this compound inhibited the binding of acetylcholine in synaptic vesicles, although yet direct application of the *in vitro* data of these regards to the events in nerve endings *in situ* was not fully accepted. Thus, the major action mechanism of 2-amino-4-phenylthiazole upon the functions of carp CNS seemed to be specifically participated with cholinergic nerve endings.

Another particular chemical change which was observed during the anesthetization was the augmentation of cAMP in carp brain. Although the explanation on the physiological consequence regarding the augmentation of cAMP in carp brain during the anesthetization was yet unavailable, it was confirmed that this compound was found to be a potent inhibitor upon cAMP phosphodiesterase activity. SUZUKI, ISHIDA and YASUMASU[*3] found that 2-amino-4-phenylthiazole also inhibited cAMP phosphodiesterase of sea urchin eggs and further that this compound did not activate adenylate-cyclase located in the membrane system of sea urchin eggs. The assumption from these experimental evidences revealed that the augmentation of cAMP in carp brain during the anesthetization should be closely related to the inhibitory action of 2-amino-4-phenylthiazole upon cAMP phosphodiesterase activity. From MCAFEE, SHORDERET and GREENGARD's findings[10] as for the function of cAMP in cervical sympathetic ganglion of rabbit, if the assumption, in which the operation of dopaminergic synapses in carp CNS also generate cAMP inside of the post synaptic membranes as in the inhibition of the cholinergic trans-

*3 A. SUZUKI, K. ISHIDA and I. YASUMASU: *Zoological Magazine*, **81**, 227 (1972).

mission in the cervical sympathetic ganglion, was allowed, the augmentation of cAMP in carp brain during the anesthetization might be derived by the operation of dopaminergic synapses which still remained active under the anesthetization and by the inhibition upon the cAMP phosphodiesterase activity at the concentration required for the anesthetization. And from the EEG activation by dopamine under the anesthetization, as previously described in this study, it might be suggested that there are apparently two kinds of dopaminergic synapses in carp CNS having respectively excitatory or inhibotory characteristic.

In view of molecular level mechanisms as for the inhibitory actions of this compound upon two species of enzymes which have quite different enzymic function, the kinetic analyses of these inhibitory actions will be important in future study lying between biochemistry and pharmacology. Further works to arrange the two radioassay methods are under progress.[11,12]

Acknowledgements

Authors gratefully thank Professor Dr. H. OZAKI, Tokyo University of Fisheries, for his guidance through out this study. Authors also thank Dr. T. YAMADA, Tokyo Metropolitan Institute of Radioisotope, for generous gift of myxamoeba strains.

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