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SYNTHESIS OF CERTAIN γ-AMINOBUTYRIC ACID DERIVATIVES AND INVESTIGATION OF THEIR EFFECTS OF LABELED [^{3}H]γ-AMINOBUTYRIC ACID ACCUMULATION BY BRAIN SYNAPTOSOMES

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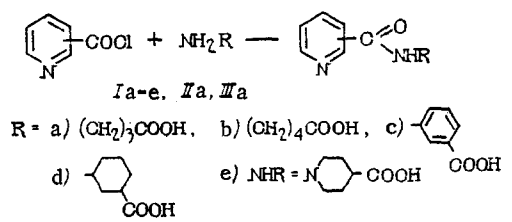
In recent years a reduction in GABA content in specific parts of the brain has been found in Parkinson's disease [1], Huntington's chorea, and convulsive states [2].

Administration of the amino acid itself rarely proves effective because of GABA's poor penetration through the blood-brain barrier. And so the search for GABA derivatives readily capable of penetrating into the brain and interacting with GABA receptors has become one way of solving the problem. At the present time there are a number of familiar GABA derivative preparations which have found application in medical practice [3]. The approach of chemically binding GABA molecules to other physiologically active substances, vitamins in particular [4], has turned out to be a promising direction in the search for medications based on GABA, and has resulted in the production of compounds which readily penetrate into the brain and which have an effect primarily on the central nervous system.

However, the actual mechanism of action of these substances remains little investigated. In this connection it seemed important to study the effect of certain N-acyl GABA derivatives displaying high neuropharmacologic activity (homopantothenic acid [5] and nicotinolyl-GABA, e.g. [6]) on the process of active GABA uptake by synaptosomes, this being one of the important mechanisms of the mediator at the presynaptic membrane level. It would be of great value to study other GABA derivatives on this model, including new compounds, and it might be used for the initial evaluation (screening) of potentially active preparations in this series.

In the present paper, we report information on the synthesis of new GABA derivatives and on a study of the effect of these compounds, as well as that of a number of previously described substances, on the process of active GABA uptake by rat cerebral cortex synaptosomes for the purpose of discovering a relationship between their chemical structure and biological activity.

Synthesis of amides of nicotinic acid (Ia-e), isonicotinic acid (IIa), and α-picolinic acid (IIIa) was carried out in accordance with the following scheme:



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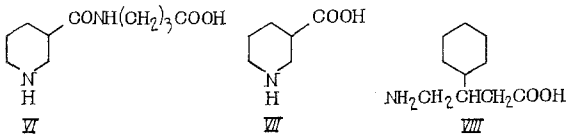
Earlier we had used condensation of nicotinic acid azide with GABA to obtain compound Ia [7]. However, this method has a number of important disadvantages related to the production of nicotinic acid azide, which is an unstable substance disposed to explosive decomposition. Schotten-Baumann condensation of the acid chloride of nicotinic acid hydrochloride with α-amino acids was described earlier [8] without experimental details. It should be noted that under these reaction conditions the acid chloride of nicotinic acid hydrolyzed considerably faster than it reacted with the α-amino acids and the yield of end-products was quite low. We found that in the case of ω-amino acids, including μ-aminobenzoic, 3-aminocyclohexane carboxylic acid, and 4-piperidine carboxylic acids, condensation with the acid chlorides of nicotinic, isonicotinic, and α-picolinic acids resulted in a relatively high yield of the amides Ia-e, IIa, and IIIa. The compounds obtained are crystalline substances which are poorly soluble in water. Hence they were converted into their sodium salts which are readily soluble in water for biological research.

In a similar manner, N-benzoyl- and μ-nitrobenzoyl-GABA (IV, V) were obtained from the acid chlorides of benzoic and μ-nitrobenzoic acid, respectively.

A number of identical absorption bands were noted in the IR spectrum of compounds Ia-e, IIa, and IIIa, which were related to the presence of common molecular structural elements (amide bond, carboxyl group, and pyridine ring). Intense bands were observed in the spectra of all of the substances in regions 3300 (NH), 1710 (COOH), 1630-1640 (amide I), 1540-1550 (amide II), and 1590-1595 cm^{-1} (pyridine ring).

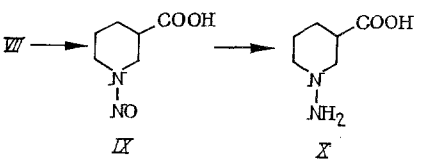
During reduction of amide Ia over 5% Rh/C its hydrogenated analog N-nipecotinoyl-GABA (VI) is produced. Under similar conditions, reduction of nicotinic acid and β-phenyl-GABA, respectively, leads to the formation of nipecotinoic acid (VII) and β-cyclohexyl-GABA (VIII).



VI VII VIII

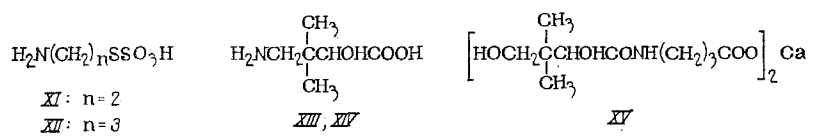
The bands at 1595 cm^{-1}, caused by the valence oscillations of the pyridine ring, and at 1710 cm^{-1} (COOH) disappear from the IR spectrum of compound VI, but absorption appears at 1580 and 1660 cm^{-1} (carboxylate anion and NH^{4+}, respectively) confirming the conversion of substance Ia into its hydrogenated analog VI. In the NMR spectrum of compound VIII, in contrast to the initial phenyl analog, there is no singlet in the weak field about δ 7.33 mmf from the protons of the phenyl ring.

The N-nitroso derivative (IX) is obtained by nitrosation of the acid of (VII) by means of nitrous acid and then cooling. Upon reduction by zinc in acetic acid, N-aminonipecotinic acid (X) is formed.



IX X

Besides the compounds mentioned above, we also investigated S-sulfocysteamine (XI), S-sulfohomocysteamine (XII), D- and L-4-amino-3,3-dimethyl-2-hydroxybutyric acids (XIII, XIV), and calcium D-homopantothenate (XV), whose syntheses were described previously [9-11].



XI: n = 2
XII: n = 3

XIII, XIV XV

EXPERIMENTAL PHARMACOLOGY

The cerebral cortices of 180-200 g male white rats were removed in accordance with the generally accepted procedure [12]. Synaptosomes were obtained by a modified technique [13]. The method of studying the process of [^{3}H]GABA uptake was similar to that described previously [14]. Protein was measured by the Lowry method. The results were analyzed statistically with computation of mean values and their confidence intervals at P = 0.05.

TABLE 1. Effect of GABA Derivatives in a Concentration of 1 mM on the Process of [^{3}H]GABA Reuptake by Rat Cerebral Cortex Synaptosomes

Name	Compound	GABA up take, % of the control
Ia	Nicotinoyl-GABA (sodium salt)	73±6
Ib	Nicotinoyl-δ-aminovaleric acid (sodium salt)	72±11
Ic	Nicotinoyl-M-aminobenzoic acid (sodium salt)	91±9
Id	Nicotinoyl-3-aminocyclohexanoic acid (calcium salt)	97±9
Ie*	Nicotinoyl-4-piperidic acid	113±13
IIa*	Isonicotinoyl-GABA	98±6
IIIa*	α-Picolinoyl-GABA	96±6
IV*	N-Benzoyl-GABA	129±13
V*	N-m-Nitrobenzoyl-GABA	105±7
VI*	Nipecotinoyl-GABA	84±4
VII	Nipecotinoic acid	20±4
VIII*	β-Cyclohexyl-GABA	87±6
X	1-Amino-3-nipecotinoic acid	34±4
XI	S-sulfoxysteamine	74±8
XII	S-sulfohomocysteamine	65±9
XIII	D-4-Amino-3,3-dimethyl-2-hydroxybutyric acid	92±9
XIV	L-4-Amino-3,3-dimethyl-2-hydroxybutyric acid	108±11
XV	Calcium D-homopantothenate (pantogam)	121±12

*Substances were dissolved by using 0.5% dimethylsulfoxide, whose addition to the incubation medium did not affect uptake (102 ± 8% of the control).

Note: 100% was taken as synaptosomal GABA uptake without the substance being tested and came to an average of 4 μmole per gram of protein in 20 min.

Results of the investigation of the effect of the compounds on the process of active [^{3}H]GABA transport by rat brain synaptosomes are presented in Table 1.

According to reports in the literature [15], nicotinic acid has no appreciable inhibitory effect on GABA uptake. As is evident from Table 1, the amides of nicotinic (Ia-e). isonicotinic (IIa), and α-picolinic (IIIa) acids do not show inhibitory activity either. A small effect can be seen with amides having an open carbon chain. Thus, Ia is moderately effective in inhibiting uptake, and acid Ib, which has one additional carbon atom, has a similar effect.

In view of the fact that nipecotinic acid (the reduced form of nicotinic acid) is a known inhibitor of the system of neuronal GABA uptake [15], it might be expected that its derivatives would display a similar effect. A moderate inhibitory effect is shown, according to our findings, by compound VI and its aminoderivative (X), i.e., compounds most resembling nipecotinic acid itself (piperidine ring stability is maintained).

It is well known that alteration of the structure of the molecule's piperidine ring leads to loss of the compounds' affinity for the GABA uptake system [15]. The intermolecular distance between the two charged centers, totaling 0.42-0.54 nm [16], as well as the pK value, are important in giving the molecule of a compound the properties of an inhibitor of the GABA uptake system. It can be assumed that modification of the nipecotinic acid molecule in the case of its derivative X is not accompanied by a significant change in these two parameters. Some decrease in the inhibitory effect of compound X can be explained by displacement of the charge of the piperidine ring's nitrogen atom caused by the influence of the amino group, which results in an increase in the distance between the molecule's charged centers. Attaching GABA to a nipecotinic acid residue apparently has a considerable effect on the properties of the compound VI obtained: Its inhibitory effect on uptake is preserved in only a moderate degree and it can be compared to the similar effect of nicotinoyl-GABA (Ia). Replacement of the piperidine ring with a benzene ring in the molecule of the GABA derivatives (IV and V) does not entail the emergence of affinity for the system of active amino acid transport.

Based on the ability of exogenous GABA to inhibit [^{3}H]GABA uptake [17], it can be stated that preservation of the free amino group is of key importance to the display of the inhibi-

TABLE 2. N-Acyl Derivatives of GABA

Compound	Yield, %	Melting point, °C	Found, %			Molecular formula	Calculated, %		
			C	H	N		C	H	N
Ia	38	213—4	57,68	5,76	13,46	$C_{10}H_{12}N_2O_3$	57,43	5,82	13,12
Ib	13,6	240—6	59,46	6,31	12,61	$C_{11}H_{14}N_2O_3$	59,56	6,62	12,89
Ic	40,2	298—9	64,46	4,13	11,57	$C_{13}H_{10}N_2O_3$	64,18	4,41	11,54
Id	48,5	264—6	62,90	6,45	11,29	$C_{13}H_{16}N_2O_3$	62,64	6,43	11,57
Ie	69,5	192—3,5	61,54	5,98	11,97	$C_{12}H_{15}N_2O_3$	61,36	6,00	11,88
IIa	37,2	230—2	57,68	5,76	13,46	$C_{10}H_{12}N_2O_3$	57,40	5,36	13,09
IIIa	30,6	112—4	57,68	5,76	13,46	$C_{10}H_{12}N_2O_3$	57,32	5,75	13,14
IV	82	48,5—50	63,34	6,73	6,53	$C_{11}H_{13}NO_3$	63,75	6,32	6,76
V	83	119—20,5	51,91	5,04	11,08	$C_{11}H_{12}N_2O_5$	52,37	4,80	11,11

tory action of GABA. In addition, the presence of nipecotinic acid's free carboxyl group is necessary for preservation of the latter's activity (reduction of the effect in the case of compound VI).

The cyclohexyl derivative of GABA (VIII), similar to phenibut, also a weak inhibitor of GABA accumulation [14], turned out to be slightly active.

Suppression of uptake was displayed by S-sulfocysteamine (XI) and S-sulfohomocysteamine (XII), which can be regarded as structural analogs of GABA and δ-aminovaleric acid. The D- and L-aminopantoic acids (XIII and XIV) had no effect on uptake. These compounds structurally resemble the alkyl- and hydroxy- derivatives of GABA which display inhibitory activity with regard to GABA uptake [14, 15]. The presence of an additional methyl group at position 3 and a hydroxyl at position 2 makes these compounds inactive, and this applies to the D form as well as the L form. Similar compounds, and especially the stereoisomers of 2-hydroxy-4-methyl-GABA had a weak inhibitory effect [19].

Calcium D-homopantothenate (XV), as well as IV, caused some stimulation of the uptake process.

EXPERIMENTAL CHEMISTRY

The IR spectra were taken on spectrophotometer UR-10 (Carl Zeiss, GDR) in vaseline oil. The PMR spectrum was recorded on a Hitachi R-20a device (Japan) (60 MHz) in D_2O.

N(α,β,γ-pyridinecarbonyl amino acids) (Ia-e, IIa, and IIIa). To a solution of 0.75 g of GABA and 0.74 g of sodium hydroxide in 10 ml of water at 0-5°C is added 1 g of the acid chloride of nicotinic acid hydrochloride in small portions. After the addition is complete, the mixture is agitated for 1 h at 5°C and allowed to warm up to 20°C in the air. The solution is acidified with concentrated hydrochloric acid to a pH of 3.0-3.5, the precipitate which has settled is filtered out, washed with cold water, and dried in a vacuum. The constants of the substances produced and the analytical results are provided in Table 2.

N-benzoyl- and N-μ-nitrobenzoyl-GABA (IV and V). These are obtained similarly by reacting benzoylchloride and μ-nitrobenzoylchloride with GABA (see Table 2).

β-Chclohexyl-GABA (VIII). In an autoclave with a 0.2-liter capacity electromagnetic agitator is placed 10 g of β-phenyl-GABA hydrochloride, 90 ml of water, and 3 g of 5% Rh/C, previously reduced with hydrogen (250°C, 3 h). Hydrogenation is carried out at 80°C and a hydrogen pressure differential of 80-70 atm. After absorption of the theoretical amount of hydrogen (about 20 min) the autoclave is cooled to 40°C and the catalyst filtered out. While stirring, a concentrated ammonia solution is added to the filtrate to a pH of 6.0-6.5, and the reaction mass is allowed to stand for 1 h at 3-5°C. The precipitate which settles out is filtered, washed with cold water, and dried in a vacuum at 70-80°C for 3 h. The yield is 8.3 g (87.0%). Found, %: C 58.68; H 10.31; N 6.62. $C_{10}H_{19}NO_2 \cdot H_2O$. Calculated, %: C 59.08; H 10.41, N 6.89.

N-(nipecotinoyl)-GABA (VI). Produced under conditions of VIII synthesis from 3 g of Ia. Yield 56%, mp 202-203°C. Found, %: C 56.10; H 8.93; N 12.62. $C_{10}H_{18}N_2O_3$. Calculated, %: C 56.05; H 8.77; N 13.08.

N-nitroso-3-piperidine Carboxylic Acid (IX). To a solution of 14 g of 3-piperidine carboxylic acid and 9.3 g of sodium nitrite in 140 ml of water over a 40-min period is added

224 ml of 3 M sulfuric acid at 20°C. Extracted with ether (5 × 200 ml), evaporated at 2-3°C in a vacuum, and obtained 11.7 g (67.5%), mp 107-110°C. Found, %: C 45.56; H 6.34; N 17.85. $C_6H_{10}N_2O_3$. Calculated, %: C 45.57; H 6.33; N 17.72.

N-amino-3-piperidine Carboxylic Acid (X). A solution of 1.2 g of substance IX in 60 ml of a 50% acetic acid solution is cooled 0-2°C, and at that temperature zinc dust is added for 10 min. The zinc is filtered out, hydrogen sulfide passed through the filtrate, and the zinc sulfide precipitate which settles out is discarded, the filtrate is evaporated, the residue is vacuum dried, treated with alcohol, and 0.6 g (52.8%) of X are obtained, mp 205-207°C. Found, %: C 49.91; H 8.40; N 18.75. $C_6H_{12}N_2O_2$. Calculated, %: C 50.00; H 8.33; N 19.39.

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