Table 3 gives the results of the investigation of LK-11 which, as the writers have found, has a stronger pharmacological action than the other derivatives of this series. In the present experiments incubation was carried out in Tris-HCl buffer, pH 7.4, and in medium containing different NA concentrations ($1 \cdot 10^{-4}$ M and $3 \cdot 10^{-6}$ M). Clearly, with an NA concentration in the medium of $1 \cdot 10^{-4}$ M, its decrease after incubation (15 min, 37°C) was 27.3% compared with the control.

On the addition of LK-11 to the medium (10^{-5} M) moderate inhibition of NA uptake was observed. Under the same conditions cocaine had a similar effect.

In medium containing the smallest NA concentration ($3 \cdot 10^{-6}$ M) its uptake by SV was practically nil in these experiments. LK-11 and cocaine in this case also had no substantial effect on the NA concentration in the incubation medium.

Like cocaine, LK-11 thus inhibits the passive uptake of NA by hypothalamic synaptic vesicles in vitro. However, this effect depends on the NA concentration in the incubation medium. If the NA concentration is deficient, liberation of bound NA from SV can be activated [5]. This process also is characteristic of hypothalamic SV, but its sensitivity to pharmacological action may be different.

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EXPERIMENTAL STUDY OF INTERACTION BETWEEN GALANTHAMINE AND MOUSE BRAIN ACETYLCHOLINESTERASE in vivo

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Injection of armin* into mice after preliminary administration of galanthamine leads to a decrease in the inhibition of brain acetylcholinesterase (ACE) induced by the reversible inhibitor. This effect is associated with the accumulation of acetylcholine and displacement of galanthamine by it from the active centers of ACE. In experiments in vivo, the competitive character of interaction between galanthamine and ACE was thus revealed.

KEY WORDS: galanthamine and armin; brain acetylcholinesterase; reversible and irreversible inhibitors.

It was shown previously that galanthamine competes with acetylcholine (AC) for the active sites on acetylcholinesterase and that an increase in the substrate concentration in vitro leads to a decrease in the inhibition of ACE by this reversible inhibitor [1, 2].

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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TABLE 1. Effect of "Primary" and "Secondary" Addition of Armin to ACE–Reversible Inhibitor Combination on True Inhibition of Enzyme by Galanthamine and Tacrine (M ± m)

	Inhibition of ACE, %					
	I series	II series	III series	IV series	V series	VI series
Galanthamine ($1 \cdot 10^{-6}$ M)	75,0±4,3	8,6±1,2	38,9±3,7	47,0±1,7*	43,0±2,1	81,0±5,1*
True inhibition	—	—	65,5±3,4	62,3±5,6	—	—
Tacrine ($1 \cdot 10^{-6}$ M)	60,0±3,1	49,3±2,5	57,1±3,3	34,5±2,2*	56,0±3,2	83,4±4,7*
True inhibition	—	—	81,8±3,4	82,8±7,5	—	—

*In these experiments ACE activity was determined relative to the activity of the enzyme remaining after primary incubation with armin.

In the investigation described below interaction between galanthamine and mouse brain ACE was studied after the subsequent injection of armin into animals in experiments in vivo.

The approach to the study of this problem was provided by the development of a technique of "double inhibition" by an irreversible inhibitor in experiments in vitro. The true degree of inhibition of ACE by reversible inhibitors was determined by consecutive addition, first of the reversible and then of the irreversible, inhibitor to the incubation medium by a method suggested previously [2, 3]. This method of addition of the irreversible inhibitor was conventionally described as "primary inhibition" by the irreversible inhibitor. In experiments in vivo it was necessary to establish the degree of inhibition of brain ACE by galanthamine after the subsequent injection of armin, i.e., when the AC concentration was increased as a result of combined inhibition of ACE by galanthamine and armin.

After addition of armin to the ACE–reversible inhibitor combination in experiments in vitro, the latter was not displaced from the active sites of the enzyme. Presumably during secondary incubation with the irreversible inhibitor ("double inhibition") the reversible inhibitor protects the ACE to the same degree as the reversible inhibitor added previously protects the enzyme against the armin added in the first place. All these assumptions require experimental verification.

EXPERIMENTAL METHOD

Mouse brain homogenate diluted with 0.14 M NaCl in the ratio of 1:19 was used as the source of ACE. In the experiments of series I 1 ml of homogenate and 1 ml of distilled water were incubated for 30 min with 0.1 ml armin in a concentration of $3 \cdot 10^{-6}$ M. In the experiments of series II, 1 ml galanthamine or tacrine in a concentration of $1 \cdot 10^{-6}$ M was added to 1 ml homogenate. In the experiments of series III, 0.1 ml armin ($3 \cdot 10^{-6}$ M) was added to a mixture of 1 ml homogenate and 1 ml galanthamine or tacrine. In series IV, after incubation of the homogenate with galanthamine or tacrine and armin, 30 min later armin was again added in the concentration used. In the experiments of series V, the concentration of armin was chosen so that its contact with the homogenates led to the same degree of inhibition of the enzyme as contact between armin in the previous concentration after preliminary addition of the reversible inhibitors. In the experiments of series VI the homogenates were incubated first with armin in the concentration chosen in series V, and then with armin in a concentration of $3 \cdot 10^{-6}$ M. The residual enzyme activity in all the experiments was determined by Hestrin's method [4] 30 min after the last addition of armin. The true value of inhibition of ACE by the reversible inhibitors was calculated by the equation suggested by the writers previously [3].

The "double inhibition" method was used to study inhibition of mouse brain ACE by galanthamine, followed by injection of armin into the animals. In the experiments of series I the animals received armin by subcutaneous injection in a dose of 0.33 mg/kg body weight. In the experiments of series II and III mice were given galanthamine by intraperitoneal injection in a dose of 4 mg/kg, followed by armin subcutaneously after 30 and 60 min in the previous dose. In the experiments of series IV galanthamine was injected into the animals, followed by armin 30 min later and again by armin after the same interval. In series V, the dose of armin which, when injected, led to the same degree of inhibition of ACE as in series II, after preliminary injection of galanthamine, was chosen in intact animals. In series VI, 30 min after injection of armin in the dose chosen in the previous series, the animals again received armin in a dose of 0.33 mg/kg. In series VII, armin was injected in the dose chosen in series V, galanthamine was injected 30 min later, and armin was again injected in a dose of 0.33 mg/kg 60 min after the reversible inhibitor. The true inhibition of ACE activity also was determined 90, 120, and 150 min after injection of galanthamine.

TABLE 2. Effect of Armin on Restoration of Mouse Brain ACE Activity after Injection of Galanthamine in a Dose of 4 mg/kg (M ± m)

Series of experiments	Inhibition of ACE, %	True inhibition of ACE by galanthamine
I—Armin (0.33 mg/kg)	90.8 ± 1.3	—
II—Galanthamine, followed after 30 min by armin (0.33 mg/kg)	39.0 ± 2.3	79.3 ± 5.3
III—Galanthamine followed after 60 min by armin (0.33 mg/kg)	59.5 ± 3.6	62.0 ± 7.5
IV—Galanthamine followed by armin twice in a dose of 0.33 mg/kg	83.5 ± 2.6*	34.8 ± 2.2
V—Armin (0.2 mg/kg)	43.2 ± 5.6	—
VI—Armin (0.2 mg/kg) followed after 30 min by armin (0.33 mg/kg)	93.7 ± 2.0*	—
VII—Armin (0.2 mg/kg) galanthamine, followed after 60 min by armin (0.33 mg/kg)	59.1 ± 3.4*	67.2 ± 5.1

*In these experiments ACE activity was determined relative to the activity of the enzyme remaining after primary incubation with armin.

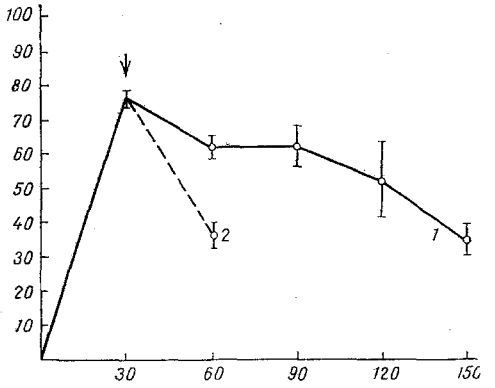

Fig. 1. Dynamics of recovery of mouse brain ACE activity after injection of galanthamine in a dose of 4 mg/kg. Abscissa, time (in min) after injection of galanthamine; ordinate, inhibition of ACE (in %). 1) Spontaneous reactivation of ACE, 2) inhibition of ACE by galanthamine after injection of armin (time of injection shown by arrow).

EXPERIMENTAL RESULTS

Secondary incubation of the ACE—reversible inhibitor—armin combination with armin did not lead to the displacement of the reversible inhibitors under the experimental conditions described. The results showed conclusively that the true inhibition of ACE by the reversible inhibitors, calculated on the basis of protection of the enzyme against the "primary" and "secondary" addition of armin, was virtually identical (Table 1).

If injected 30 and 60 min before the primary dose of armin, galanthamine inhibited the ACE of the animals' brain by 79.3 and 62% respectively (Table 2, series II and III). If a second injection of armin was given (series IV) galanthamine inhibited the activity of the enzyme by only 34.8% 60 min after its injection. If, after a primary injection of armin, galanthamine was given 60 min before the second injection of armin (series VII), a protective effect equally strong as in the experiments of series III was observed.

The experiments thus showed that after injection of armin galanthamine is displaced from the active sites of ACE. By 60 min after injection of galanthamine, the degree of inhibition of the enzyme activity corres-

ponded to the inhibition found 150 min after the use of the reversible inhibitor in the presence of spontaneous reactivation of ACE (Fig. 1). The fall in the level of ACE inhibition by galanthamine after treatment with armine took place more rapidly and was more like a "break-away" than a gradual liberation. This "break-away" is most likely linked with a considerable or indeed almost total depression of ACE as a result of the successive action of the reversible and irreversible inhibitors. AC accumulating as a result of this inhibition of the enzyme can evidently displace galanthamine rapidly from the active sites of ACE and reduce the inhibitory effect.

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EFFECT OF PHENFORMIN ON LIPID AND CARBOHYDRATE METABOLISM IN PREGNANT RATS

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A decrease in the blood levels of cholestrol, phospholipids, and free fatty acids was observed in rats receiving the biguanide phenformin (5-25 mg dailyby mouth) from the first to eighth day of pregnancy, but no developmental anomalies of the fetuses or placenta were found. The acceptability of biguanide administration during pregnancy is discussed.

KEY WORDS: pregnancy; phenformin; biguanides; lipid and carbohydrate metabolism.

The similarity between the mechanisms of the changes in energy homeostasis during aging, atherosclerosis, adiposity, several malignant neoplasms, and some other diseases suggests that the same preventive and therapeutic measures could prove useful in all these states [2-4]. Disturbances of lipid and carbohydrate metabolism during normal pregnancy, manifested primarily as hyperlipidemia, are very similar to those observed during aging, and whereas according to some workers they are "physiological" [10, 17], they evidently are not without their effects on the body [1, 4, 19]. The search for methods of eradicating these disturbances or confining them within bounds accordingly merits the closest attention. Recently the antidiabetic biguanides, which include phenformin (Dibotin [5, 7, 18], have become widely used as agents to normalize various disturbances of lipid and carbohydrate metabolism. As long ago as in 1963, Sterne [15] successfully used a biguanide (methformin) during pregnancy to control diabetes mellitus. In 1974, Notelovitz [11] expressed the view that the use of phenformin in diabetes of pregnancy was acceptable. However, the more extensive use of biguanides in pregnant women is prevented by the lack of knowledge of the effect of these preparations on the metabolism and course of normal pregnancy.

In the investigation described below an attempt was made to remedy this deficiency.

EXPERIMENTAL METHOD

Female rats weighing initially 160-180 g were used.

On the 16th-17th day the animals were mated and the discovery of spermatozoa in the vaginal contents of the females next day indicated the first day of pregnancy. The pregnant females were kept in individual cages until the end of the experiment. Some rats from the 1st or 8th day of pregnancy received 5 or 25 mg phenformin

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