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BRIEF COMMUNICATION

Chronic Treatment With Amitriptyline Alters the GABA-Mediated Uptake of $^{36}Cl^-$ in the Rat Brain

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MALATYNSKA, E., M. L. GIROUX, S. C. DILSAVER AND S. B. SCHWARZKOPF. *Chronic treatment with amitriptyline alters the GABA-mediated uptake of $^{36}Cl^-$ in the rat brain.* PHARMACOL BIOCHEM BEHAV **39**(2) 553–556, 1991.—Amitriptyline inhibits the GABA-mediated uptake of $^{36}Cl^-$ in membrane vesicles prepared from the cerebral cortices of drug-naive and saline-treated rats. In contrast, chronic in vivo treatment with amitriptyline affects an increase in the GABA-stimulated uptake of chloride ions in its presence. The benzodiazepine receptor antagonist ZK 93426 blocks the capacity of amitriptyline to augment the uptake of $^{36}Cl^-$ by 30 μM GABA. There is a possibility that there are two distinct effects of amitriptyline's action in the rat forebrain. The first is evident in vesicles from drug-naive animals and the second only after chronic treatment with this antidepressant. The authors discuss the pertinence of this finding to the mechanism of action of amitriptyline.

Amitriptyline Antidepressants Benzodiazepine-$GABA_A$ receptor complex $^{36}Cl^-$ uptake Cerebral cortex ZK 93426

RECENT in vitro studies using membrane vesicles obtained from the rat brain indicate that there are effects of antidepressants on the $GABA_A$ receptor chloride-ionophore complex. These drugs can inhibit the GABA-mediated uptake of chloride ions (5), [^{35}S]t-butylobicyclophosphorothionate binding (10) and the GABA-stimulated uptake of $^{36}Cl^-$ in membrane vesicles obtained from the forebrain of drug-naive rats (6). However, reports of the effect of chronic treatment with antidepressants on the functional characteristics of the complex have yet to be published. This is particularly interesting given growing evidence

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for an involvement of GABAergic systems in the pathophysiology of depression.

The $^{36}Cl^-$ uptake assay developed by Harris and Allan (1) allows in vitro assessment of the functional consequences of agents acting on the $GABA_A$ receptor chloride-ionophore complex. This method is sufficiently sensitive to allow detection of allosteric interactions of barbiturates (2) and benzodiazepines (9) on GABA-stimulated chloride-ion conductance.

The therapeutic effects of the tricyclic antidepressants generally become evident only following 2–6 weeks of treatment (4). This delay suggests gradual alteration of brain mechanisms. The study reported here was designed to answer the question of whether chronic in vivo treatment with amitriptyline hydrochloride (AMI) alters the function of the $GABA_A$ receptor complex using GABA-stimulated $^{36}Cl^-$ uptake as a biochemical endpoint.

METHOD

Two groups of six adult (200–220 g), male Sprague-Dawley rats were treated with AMI for 14 or 21 days. The drug (Sigma Chemical Co.) was diluted to a concentration of 10 mg/ml using normal saline. Each animal received AMI at a dose of 10 mg/kg or 10 ml/kg of vehicle IP at 9:00 a.m. and 5:00 p.m. for either 7 or 14 days. The animals were sacrificed 36 hours following the last injection. This lag is sufficient to allow the elimination of AMI from rat cortical tissue (8). The cerebral cortices were removed for use in the $^{36}Cl^-$ flux assay. The method for determination of GABA-mediated $^{36}Cl^-$ uptake is described elsewhere (7,9).

Membrane vesicles were prepared from cortical tissue by homogenization and centrifugation using a buffer conducive to the measurement of chloride flux. The buffer consisted of 145 mM NaCl, 5 mM KCl, 1.0 mM $MgCl_2$, 10 mM D-glucose, 1.0 mM $CaCl_2$, and 10 mM HEPES adjusted to pH 7.5 with TRIS base. Samples (200 μl aliquots) of the vesicle preparation were added to 1.8 ml (1:10 dilution) of the buffer. The vesicles were preincubated for 10 min at 30°C in the presence of log increments AMI (10^{-9}–10^{-4} M), AMI plus 1 μM ZK 93426, a β-carboline antagonist (kindly provided by Berlex Laboratories), or saline (30 μM GABA-stimulation control).

The uptake of $^{36}Cl^-$ was initiated by the addition of 200 μl of buffer containing 60 μM GABA (final concentration 30 μM) and 0.2 μCi of $^{36}Cl^-$ (New England Nuclear). GABA-mediated chloride uptake was terminated after 3 s by the addition of ice-cold buffer followed by filtration through Whatman GF/C glass fiber filters. Nonspecific $^{36}Cl^-$ flux was measured in incubates containing 100 μM bicuculline in the absence of GABA. Specific conductance was defined as the difference in chloride uptake between GABA-stimulated (control) and bicuculline-treated samples. The results are expressed as the percent of GABA-stimulated $^{36}Cl^-$ uptake where the value for stimulation produced by 30 μM GABA alone is defined as 100%.

Data were examined by two-way analysis of variance with repeated measures using the Statistical Analysis System (SAS) General Linear Model procedure.

RESULTS

Preincubation of membrane vesicles obtained from saline- and AMI- (14 and 21 days) treated rats with increasing concentrations of AMI resulted in different responses of GABA-stimulated $^{36}Cl^-$ uptake in the various treatment groups (Fig. 1). These responses varied from inhibition of GABA-stimulated $^{36}Cl^-$ uptake in cortical tissue from saline-treated rats to stimulation of $^{36}Cl^-$ uptake in tissue from rats treated with AMI for 21 days. GABA-mediated chloride uptake was stimulated by lower (10^{-8} and 10^{-7} M) and inhibited by higher (10^{-6} and 10^{-4} M) concentrations of AMI in membrane vesicles from the rats treated with AMI for 14 days.

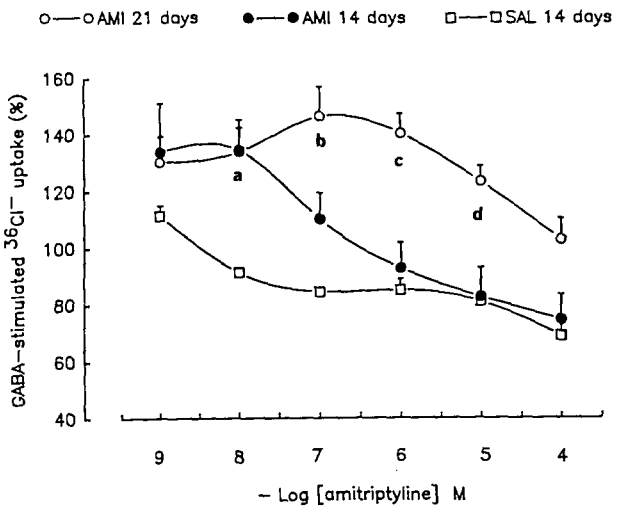

FIG. 1. The results of the chronic treatment of rats with AMI on $^{36}Cl^-$ uptake in membrane vesicles from rat cerebral cortices in presence of AMI in assay medium. The value of 100% is the stimulation of $^{36}Cl^-$ uptake produced by 30 μM GABA alone. The figure shows the means and standard errors of the values for chloride uptake by treatment group and concentration of amitriptyline. The values marked by letters differ significantly as determined by using a two-way ANOVA followed by Student-Newman-Keuls test (a) ANOVA $p=0.004$, amitriptyline (AMI) 21-day = AMI 14-day > SAL (saline-treated group), (b) $p=0.0014$, AMI 21-day > AMI 14-day > SAL, (c) $p=0.0024$, AMI 21-day > AMI 14-day = SAL, (d) $p=0.0094$, AMI 21-day > AMI 14-day = SAL.

Repeated measures analysis of variance revealed significant main effects of treatment group ($p<0.0001$) and AMI concentration ($p<0.0005$). The main effect of treatment was lowest in the saline group, intermediate in magnitude in tissue obtained from animals treated for 14 days and greatest following 21 days of treatment. The significant effect of concentration is manifested by decreasing values of $^{36}Cl^-$ uptake with increasing concentrations of AMI in all three treatment groups. Examination of group differences at each concentration of AMI showed significant differences between the saline-treated sample and those groups treated with the antidepressant for 14 and 21 days at the concentrations of 10^{-8} to 10^{-5} M. GABA-stimulated $^{36}Cl^-$ uptake was enhanced in membrane vesicles from 21-day AMI-treated rats compared with saline-treated rats ($p<0.01$) at each of these concentrations. GABA-stimulated $^{36}Cl^-$ uptake was also enhanced ($p<0.01$) at concentrations of 10^{-7} to 10^{-5} M in membrane vesicles from those animals treated for 21 relative to 14 days. Fourteen days of treatment with AMI significantly enhanced GABA-stimulated $^{36}Cl^-$ uptake at concentrations of 10^{-8} and 10^{-7} M relative to the saline group ($p<0.01$).

ZK 93426 blocked the capacity of 14 days of treatment with AMI to enhance the GABA-stimulated uptake of $^{36}Cl^-$ at concentrations of AMI of 10^{-8} and 10^{-7} M ($p<0.025$) (Fig. 2). ZK 93426 also produced a trend ($p<0.1$) toward potentiation of the inhibitory effect of AMI at concentrations of 10^{-6} to 10^{-5} M AMI in vitro. The main effect of group was highly significant in those samples in which chloride uptake was measured in the presence of AMI as opposed to AMI plus ZK 93426 ($p<0.0001$).

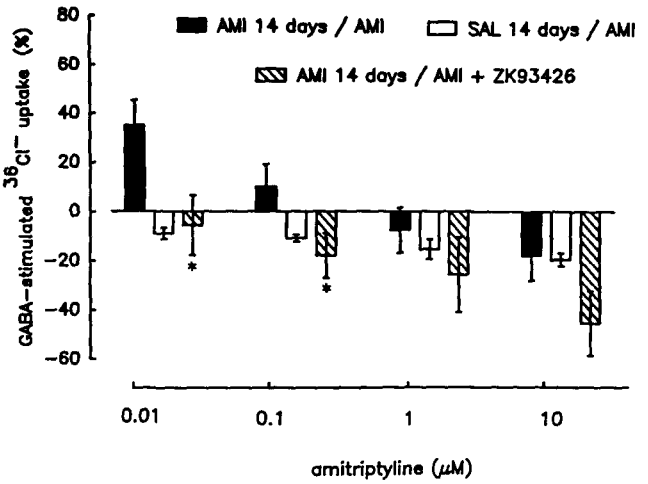

FIG. 2. The in vitro interaction of benzodiazepine receptor antagonist ZK 93426 with AMI in membrane vesicles from the cerebral cortex of rats treated with AMI for two weeks. 0 = the degree of stimulation of chloride conductance produced by 30 μM GABA alone. The positive and negative values on y axis represent the % of stimulation or inhibition of GABA-mediated chloride uptake respectively. The signifcant ($p<0.025$) blockade by ZK 93426 of the stimulatory effect of AMI in the vesicles preincubated in the presence versus absence of ZK 93426 is marked as (*).

DISCUSSION

Chronic in vivo treatment with AMI reversed the response of the $GABA_A$ receptor chloride-ionophore complex to this antidepressant in vitro. There was a significant increase in the maximal level of GABA-stimulated $^{36}Cl^-$ uptake following chronic treatment with AMI relative to the saline-treated control rats when AMI was present in the assay medium.

It is difficult to define the mechanism responsible for this in vivo alteration. The complex concentration-response curves describing the uptake of $^{36}Cl^-$ (Fig. 1) are not consistent with a single dose-response relationship. AMI appears to have two effects. The data from the saline-injected control animals suggests that increasing concentrations of AMI reduces the GABA-stimulated uptake of $^{36}Cl^-$ uptake in vitro. This result is in agreement with previous studies in which the effect of tricyclic and nontricyclic antidepressants on the GABA-mediated uptake of chloride was measured using the same assay (6). Squires and Saederup (10) showed that AMI does not reverse the inhibitory action of GABA on [^{35}S]t-butylobicyclophosphorothionate binding. Thus AMI does not have a sufficient effect on the $GABA_A$ receptor chloride-ionophore complex to reduce GABA-related activity in all types of assays. The activity of AMI may depend on the experimental condition used. It should be noted that other antidepressants, e.g., mianserine and amoxapine which more potently inhibit GABA-stimulated $^{36}Cl^-$ uptake (3,6) were also found to be active by Squires and Saederup (10).

The essential difference between the chronic AMI and saline control groups was an AMI-associated increase in maximal GABA-stimulated chloride conductance following chronic in vivo treatment with this particular tricyclic antidepressant. The maximal effect is higher for the animals treated with AMI for three rather than just two weeks. However, increasing in vitro concentrations of AMI decreased $^{36}Cl^-$ uptake in tissue taken from the AMI-treated animals. These observations suggest that AMI has two actions when chloride uptake is measured in tissue taken from the chronic treatment groups.

The capacity of increasing concentrations of AMI to reduce $^{36}Cl^-$ uptake is one of the effects of AMI on the $GABA_A$ chloride-ionophore complex. This concentration-dependent effect was seen in all treatment groups. The reduction of GABA-stimulated $^{36}Cl^-$ uptake produced by increasing the concentration of AMI may be due to the same mechanism affecting inhibition of GABA-stimulated chloride conductance in membrane vesicles prepared from the tissue of drug-naive animals.

A second effect of the tricyclic is suggested by the data obtained after two and three weeks of treatment with AMI in vivo in the presence of in vitro concentrations of AMI of 10–100 nM, and 10 nM–10 μM respectively. GABA-stimulated chloride conductance measured at these concentrations was markedly increased by prior treatment with AMI. Thus chronic treatment of the animals with AMI results in augmentation of GABA-stimulated chloride conductance. This enhancement of GABA-stimulated $^{36}Cl^-$ uptake is dramatically different from that seen in the saline-treated animals in previous studies (6) with untreated animals. This second effect of AMI becomes more pronounced with increasing duration of in vivo treatment with the antidepressant. The time course is similar to that required for the treatment of patients with depressive illness (4).

The ability of the β-carboline antagonist ZK 93426 to block the augmentation of GABA-stimulated chloride conductance produced by 14 days of in vivo treatment with AMI suggests a possible explanation for the second of AMI's two effects. ZK 93426 acts as a pure benzodiazepine receptor antagonist under the conditions used in the $^{36}Cl^-$ uptake assay. ZK 93426 has no intrinsic activity and fully blocks the activity of β-carboline agonists at the concentration used in this report (7). ZK 93426 did not reduce AMI's inhibition of GABA-stimulated chloride conductance measured in membrane vesicles from drug-naive animals (3). ZK 93426 reduced AMI-induced enhancement of GABA-gated chloride conductance to the level of the inhibitory effect produced by AMI in the saline-treated animals. These results indicate that the second mode of AMI's activity resembles that of a β-carboline agonist.

The monoamine hypotheses of antidepressant drug action have been criticized because there is no relationship between the time course for the onset of blockade of monoamine reuptake and the onset of the antidepressant effect of these agents (11). It is reasonable to anticipate that chronic biochemical effects of the antidepressants are related to their therapeutic efficacy. The second mode or effect of AMI's activity is consistent with this expectation. However, it is premature to attribute therapeutic effects to an enhancement of GABA-stimulated chloride conductance. A variety of somatic treatments are effective in the treatment of depression. Definite conclusions on the mechanism of action of these treatments must await study of each of these alternatives. Such investigations are now underway.

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