Mechanisms of Modulation of GABA-Induced Currents in Isolated Cerebellar Cells by Tacrine

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Tacrine, when applied by the concentration clamp technique to isolated Purkinje cells from rat cerebellum, dose-dependently reduced the amplitude of GABA-activated chloride currents recorded by the patch clamp technique. Half-maximal inhibition (IC_{50}) of currents activated by 2 μM GABA was observed at a tacrine concentration of 107 μM. Tacrine produced a right shift of the dose-response curves for GABA-induced currents without affecting their peak amplitudes. It is suggested that suppression of GABA-induced currents caused by tacrine can not be attributed to its interaction with the benzodiazepine site of the $GABA_A$ receptor.

Key Words: *tacrine; $GABA_A$-receptors; cerebellum; Alzheimer disease; voltage clamp*

Interest in the research of neurochemical effects of tacrine (9-amino-1,2,3,4-tetrahydroacridine) has been stimulated by its alleviating action on cognitive dysfunction in Alzheimer disease [13]. The mechanisms of this effect have not yet been completely understood. Since tacrine is a cholinesterase inhibitor acting within the central nervous system, and its therapeutic effect can be attributed to attenuation of the cholinergic deficit in Alzheimer disease [5]. However, other anticholinesterase agents were inefficient in Alzheimer disease [3], which suggests that the therapeutic action of tacrine is mediated by other mechanisms. Tacrine reverses morphine- and magnesium chloride-induced coma [11], alleviates narcotic withdrawal symptoms [12], and reduces the duration of ketamine-induced anesthesia [2]. In hippocampal slices, tacrine increased the pop-spike amplitude in a concentration of 0.5 μM, but decreased it in higher concentrations (10-50 μM) [1].

Such a great variety of medical and physiological effects of tacrine is probably related to a broad spectrum of its pharmacological activity. Apart from anticholinesterase activity, tacrine acts as an antagonist of muscarinic M_1 and M_2 receptors [9] and a blocker of voltage-gated potassium and sodium channels [10]. Tacrine produces a potential-dependent blockade of NMDA [8] and $GABA_B$ receptor potassium channels [6], inhibits binding of flunitrazepam to benzodiazepine (BZ) receptors and GABA binding to $GABA_A$ receptors [4].

In this study we investigated the mechanisms of tacrine interaction with $GABA_A$ receptors by recording the whole-cell currents activated by GABA application in isolated Purkinje cells from rat cerebellum.

MATERIALS AND METHODS

Experiments were carried out on isolated Purkinje cells dissociated from rat cerebellar slices by vibration [14]. Cerebellar slices were incubated at room temperature in a solution containing (in mM): 124 NaCl, 5 KCl, 1.3 $CaCl_2$, 1.5 $MgCl_2$, 1.3 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose and saturated with carbogen (95% O_2+ 5% CO_2). Cell isolation and recording of electrical activity were performed in a solution containing (in mM): 150 NaCl, 5 KCl, 2.7 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES. Borosilicate micropipettes (2-4 mΩ resistance) were filled with a solution of the following composition (in mM): 140 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 5 EGTA, 10 HEPES, and 4 Na_2-ATP.

When high GABA concentrations were applied to activate the currents, 100 mM KCl in the intracellular

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solution was replaced by $KMeSO_3$ to minimize possible error caused by voltage drop through the uncompensated series resistance. In some experiments KCl was replaced by CsCl. A modified fast perfusion technique was used for drug application [15] which allowed the solution around the recorded neuron to be changed in approximately 40 msec. The currents were recorded at room temperature.

The concentration-response relationships for GABA-induced currents were described by the following equation: $A=1/[1+(EC_{50}/GABA)^n]$, where $A=I_{GABA}/I_{GABA(50\mu M)}$ is a relative GABA-induced response; EC_{50} is GABA concentration producing a half-maximal response, and n is the Hill coefficient. To quantify the inhibitory effect of tacrine on the currents induced by a constant GABA concentration the following equation was used: $B=1-[1/1+(IC_{50}/tacrine)^n]$, where $B= I_{GABA+tacrine}/I_{GABA}$ is the degree of tacrine-induced blockade of GABA-induced currents. The responses were normalized to the amplitudes of currents activated by various GABA concentrations in the absence of tacrine; IC_{50} is the tacrine concentration producing a half-maximal inhibitory effect.

RESULTS

In all tested neurons, application of 1-50 μM GABA at a holding potential of -70 mV induced an inward current. The amplitude of this current increased with increasing GABA concentration. This current was blocked by bicuculline, decreased when intracellular Cl^- was replaced with $MeSO_3^-$, and disappeared with of intracellular Cl^- replacement with F^-. Therefore, the recorded currents were mediated by GABA receptors coupled with chloride channels. In the majority of the experiments, 2 μM GABA was applied for a 1-sec periods with 30-sec intervals between applications. Under these conditions, stable response to GABA persisted for 30 min and over.

Coapplication of GABA and tacrine produced a dose-dependent decrease in the amplitude of GABA-activated currents in all tested neurons (Fig. 1, *a*). At a GABA concentration of 2 μM the inhibitory action of tacrine was characterized by IC_{50}=107.4±3.9 μM and a Hill coefficient of 2.0±0.15 (n=4). Tacrine-induced inhibition was decreased with increasing the GABA concentration. In the presence of 5 μM GABA, the IC_{50} for tacrine increased to 184.0±11.6 μM with a Hill coefficient of 1.9±0.2 (n=4), and at 10 μM GABA to 383.1±7.8 μM with a Hill coefficient of 1.8±0.1 (n=4) (Fig. 1, *b*). The effects of tacrine developed rapidly (changes of the response amplitude were observed immediately after combined application of GABA and tacrine) (Figs. 1, *a*; 2, *b*) and easily reversible. Tacrine did not affect the kinetics of GABA-activated currents and their desensitization at a relatively high GABA concentrations (Figs. 1, *a*; 2, *a*, *b*). The effects of the drug did not depend on the holding voltage (data not shown).

Tacrine had no effects on currents induced in the same neurons by application of 100 μM kainate, an agonist of AMPA (α-amino-3-hydroxy-5-methyl-4-izoxasole propionic acid)/kainate receptors, or by 100 μM glycine, which is indicative of a relative specificity of tacrine interaction with GABA receptors.

To analyze the mechanism of tacrine-induced inhibition of GABA-activated currents, we compared the concentration-response relationships for GABA-activated currents under control conditions and in the presence of 100 μM tacrine (Fig. 2) In the control, EC_{50} for GABA was 3.95±0.13 μM and the corresponding Hill coefficient was 2.4±0.15. In the presence of 100 μM tacrine, EC_{50} for GABA increased to 6.62±0.33 μM (p<0.05, Fig. 2, *c*), while the Hill co-

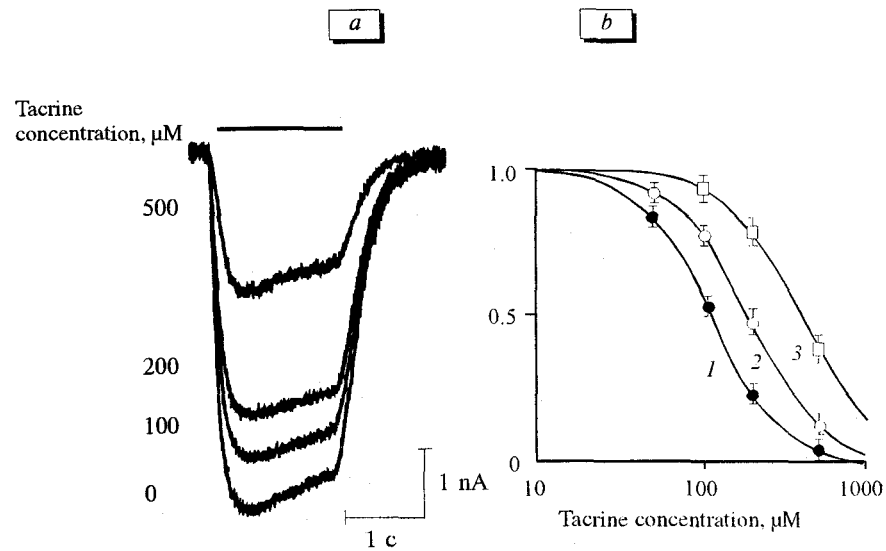

Fig. 1. Tacrine blocks Cl^- current induced by rapid application of GABA in cerebellar Purkinje cells. *a*) changes in current amplitudes after coapplication of 10 μM GABA and increasing concentrations of tacrine. Here and in Figs. 2 and 3: application period is marked by a horizontal line above the curves. Holding potential is -70 mV; *b*) dose-response curves for the inhibitory effect of tacrine on currents induced by 2 (*1*), 5 (*2*) and 10 (*3*) μM GABA. Ordinate: relative changes in the mean amplitude of GABA-activated currents. The degree of tacrine inhibition decreased with increasing GABA concentrations.

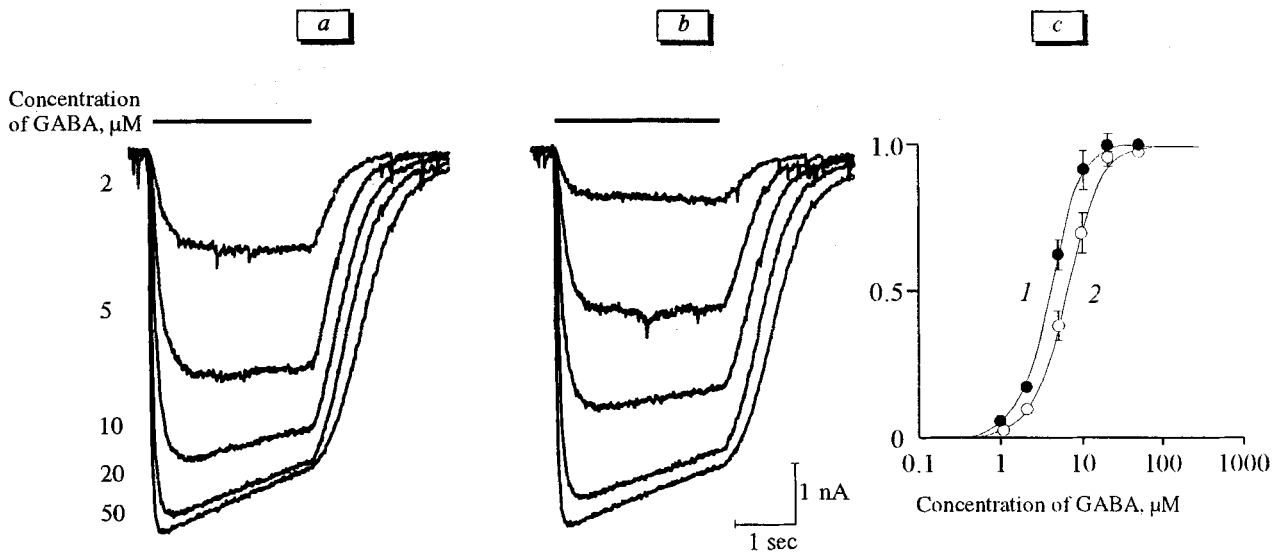

Fig. 2. Attenuation of tacrine inhibitory effects with increasing GABA concentrations. *a*) currents induced by increasing GABA concentrations; *b*) responses to the same concentrations in the presence of tacrine; *c*) dose-response curves for GABA-activated currents in the absence (*1*) and presence of 100 μM tacrine (*2*). Tacrine causes a right shift of the dose-response curve for GABA.

efficient remained unaffected (2.0±0.17, n=6). Tacrine caused a right shift of the dose-response curve for the amplitude of GABA-induced currents (Fig. 2, *c*) without affecting the peak currents induced by GABA application (Fig. 1, *a, b*).

The increase in IC_{50} with increasing GABA concentration, the right shift of the dose-response curve, and independence of the tacrine effects on the membrane potential indicate that tacrine is a competitive $GABA_A$ receptor antagonist. However, it remains unclear whether tacrine directly interacts with the GABA binding site or allosterically inhibits GABA binding.

The $GABA_A$ receptor-channel complex includes several regulatory sites modulated by various bioactive compounds, such as BZ, barbiturates, and steroids. It was reported that tacrine inhibits the binding of BZ to $GABA_A$ receptors [4]. To test the possibility of direct interaction of tacrine with the BZ binding site, we investigated its effects on currents induced by GABA alone (control) and in combination with diazepam (2 μM GABA+1 μM diazepam potentiated, Fig. 3) Tacrine in a concentration of 100 μM diminished GABA-activated currents to 0.53±0.02 (n=9), while diazepam enhanced it up to 1.81±0.12 of the control (n=6). In the presence of 1 μM diazepam, tacrine (100 μM) reduced the current to 0.56±0.05 of the control value (n=5), *i. e.* to the same extent as under control conditions. Tacrine did not affect the kinetics of diazepam effects (Fig. 3). The BZ receptor antagonist flumazenil in a concentration of 5 μM completely abolished the effects of diazepam, but did not affect tacrine-induced inhibition of GABA-activated currents. These findings

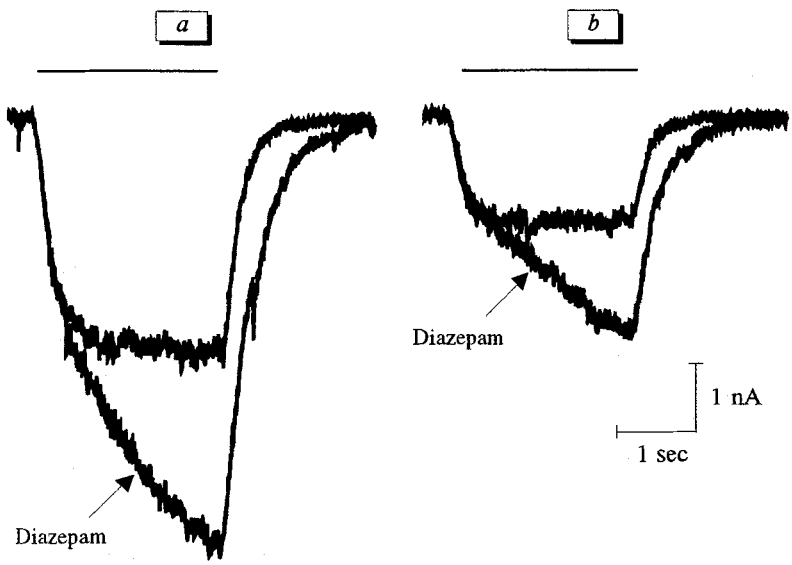

Fig. 3. Tacrine-induced inhibition of GABA-induced responses potentiated by diazepam. Single recording at a holding potential of -70 mV. *a*) potentation of GABA-induced current after coapplication of 2 μM GABA, and 1 μM diazepam; *b*) tacrine-induced block of GABA-activated current potentiated by 1 μM diazepam. Tacrine concentration is 100 μ.

do not support the hypothesis on tacrine interaction with the BZ binding site on the $GABA_A$ receptor complex.

Thus, our data showed that tacrine is a competitive antagonist of $GABA_A$ receptors. Its inhibitory effect on the GABA-mediated responses manifests itself at concentrations far surpassing than the effective serum concentrations (0.1-1.0 μM) revealed in clinical studies [7,13]. This implies that tacrine interaction with $GABA_A$ receptors is hardly involved in the therapeutic effects of this drug in Alzheimer disease. However, these results can contribute to understanding of the mechanisms of action of tacrine and related drugs. It can not be excluded that the weak blockade of $GABA_A$ receptors by tacrine in combination with its effect on other receptors and ion channels contributes to a wide pharmacological spectrum of this drug.

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