Activation of subconductance states by γ-aminobutyric acid and its analogs in chick cerebral neurons

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Abstract. The action of γ-aminobutyric acid (GABA) and the GABA\(_A\)-receptor agonists muscimol, isoguvacine and 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP) were studied at the single-channel level in outside-out membrane patches from cultured chick cerebral neurons. All agonists activated channels with multiple-conductance states. The main-state conductance activated by all agonists had a value around 26 pS in symmetrical TRIS/Cl solutions. Subconductance states of around 13 pS and 18 pS were seen with application of each agonist. Muscimol and isoguvacine tended preferentially to activate subconductance states. Gating by all agonists was complex. Open-time distributions for main-state activity gated by GABA, isoguvacine and THIP were best described by the sum of two exponential curves with similar time constants. Muscimol-gated activity was best described by the sum of three exponentials indicating the presence of an additional longer open state. These results indicate that certain GABA\(_A\)-receptor agonists are capable of preferentially activating subconductance states.

Key words: Cultured chick cerebral neurons — Cl channel — GABA — Muscimol — Isoguvacine — THIP — Multiple conductance states — Subconductance states

Introduction

The principal inhibitory neurotransmitter in the vertebrate brain is γ-aminobutyric acid (GABA). The bicuculline-sensitive GABA\(_A\) receptor, which mediates fast inhibitory neurotransmission, is coupled to a chloride-selective ion channel and receptor activation leads to an increase in chloride permeability. Single-channel studies of GABA-gated chloride channels have shown that GABA activates multiple-conductance states (Hamill et al. 1983; Weiss et al. 1988). Other ion channels, such as voltage-dependent (Bosma 1989) and glycine-activated (Bormann et al. 1987) chloride channels, or ligand-gated cation channels (Hamill and Sakmann 1981; Cull-Candy and Usowicz 1987; Jahr and Stevens 1987) also show multiple-conductance states. The functional significance of multiple-conductance states is unclear and selective modulation of these states has not been reported.

In addition to GABA, other ligands for the GABA\(_A\) receptor include the naturally occurring compound, muscimol, obtained from the mushroom *Amanita muscaria* (Krogsgaard-Larsen et al. 1979), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP), a rigid bicyclic analog of muscimol, and isoguvacine, a synthetic GABA agonist (Krogsgaard-Larsen and Falch 1981). The properties of these agonists have been examined in cultured mouse spinal neurons using fluctuation (noise) analysis (Barker and Mathers 1981). THIP and isoguvacine opened ion channels whose lifetime was approximately half that of GABA whereas muscimol-activated channels had lifetimes double those of GABA-gated channels (Barker and Mathers 1981). The elementary conductance did not differ significantly among agonists. Studies of single-channel currents activated by nicotinic agonists have also indicated that differences in gating properties and not single-channel conductance account for the variation among compounds (Gardner et al. 1984).

Previous electrophysiological investigations from this laboratory have described the properties of GABA-gated chloride channels in cultured chick cerebral neurons (Weiss 1988; Weiss et al. 1988). These studies indicated the presence of multiple-conductance states and complex gating of the GABA-gated chloride channel (Weiss 1988; Weiss and Magleby 1989). Given the inability of fluctuation analysis to resolve the complexities of ion channel activity we have compared the biophysical properties of chloride channels gated by GABA, THIP, muscimol and isoguvacine at the single-channel level. The present results indicate that GABA agonists activate multiple-conductance levels similar to those of the natural ligand GABA.
In addition, muscimol and isoguvacine increase the occurrence of subconductance levels compared to GABA.

A preliminary account of some of these results has appeared (Mistry and Hablitz 1989).

Materials and methods

Cell culture. Chick cerebral neurons were cultured as described previously (Thampy et al. 1983). Cerebral hemispheres from white leghorn chicks at embryonic stage 34 were dissected out and placed in Dulbecco's modified Eagle medium (DMEM) containing penicillin (100 units/ml) and streptomycin (100 μg/ml). The hemispheres were then dissociated by extrusion through a nylon mesh (44 μm pore) and plated onto polylysine-coated plastic coverslips contained in a 35-mm plastic dish. Neurons were incubated in a humidified atmosphere with 10% CO₂ at 37°C. The culture medium consisted of DMEM supplemented with 2% fetal calf serum and 8% donor horse serum. Neurons were studied after 5–25 days in vitro.

Single-channel recordings. Outside-out patches were obtained at room temperature using standard techniques. The bath solution contained (in mM) NaCl 140, KCl 3, CaCl₂ 2, MgCl₂ 1, HEPES 10, glucose 10 (pH 7.3, adjusted with NaOH). The internal solution contained in the patch pipettes consisted of (in mM) TRIS/Cl 145, HEPES 10, glucose 10, EGTA 11, CaCl₂ 1 (pH 7.3; NaOH). After procurement of the outside-out patch, the patch was maneuvered close to the mouth of a large (200 μm diameter) perfusion pipette. This perfusion pipette was connected to four reservoirs, which contained agonists dissolved in an external TRIS solution composed of (in mM): TRIS/Cl 140; CaCl₂ 2, MgCl₂ 1, KCl 3, glucose 10, HEPES 10, pH 7.3 (NaOH).

Single-channel currents were recorded with an Axopatch 1 B amplifier and stored on videotape using a Neurocorder DR-384. For analysis, single-channel data were filtered at 1–2 kHz with an eight-pole low-pass Bessel filter and consecutively digitized at 8–12 kHz on a PDP-11/73 computer. Single-channel current amplitudes were measured manually using cursors on records that contained long openings, as described previously (Weiss et al. 1988). Single-channel conductances and reversal potentials were obtained from current-voltage (I–V) plots using linear regression analysis. Openings of agonist-gated channels to more than one distinct conductance level were observed. In order to quantify these observations, total-amplitude histograms were created and fitted with multi-component gaussian functions. The percentage of time that the channel spends in a particular conductance state was calculated from these functions. Open-time distributions were determined for the main conductance state using the methods described by Weiss (1988). Histograms were fitted with a sum of exponentials (non-linear least-squares method) using DISCRETE (Provencher 1976) starting at 0.3–0.4 ms. More complex fits to the data (i.e., those containing more exponential components) were accepted only if the probability that this solution was better exceeded 0.98.

Agonists. Stock solutions of agonists (GABA 1 mM, muscimol 0.25 mM, THIP 1 mM, isoguvacine 0.5 mM) were prepared and frozen in 1-ml aliquots. Prior to each experiment, the stock solutions were diluted with the external TRIS/Cl solution to achieve the required concentration. The concentrations of agonists used to evoke channel activity in outside-out patches were 0.5 μM or 1 μM GABA, 0.15–0.25 μM muscimol, 0.5 μM or 1 μM isoguvacine and 2–5 μM THIP.

Results

Agonist-gated single-channel currents

GABA. Application of GABA (500 nM or 1 μM) resulted in an increased frequency of occurrence of single-channel currents and the initiation of bursting activity. In those patches in which there was frequent spontaneous activity, GABA evoked a large increase in activity with several channels being activated. In these patches, multiple openings were frequent. However, in other patches in which spontaneous activity was infrequent GABA caused an increase in the frequency of occurrence of events and the distinct appearance of bursting. Multiple simultaneous openings were rare in these patches. This report is based on data obtained from the latter type of patches.

As found for spontaneous events, the predominant (main) conductance level was around 26 pS for all agonist activity. Examples of openings to the main conductance state in response to GABA, isoguvacine and muscimol are shown in Fig. 1 A, C, E, respectively. The corresponding I–V relationships are illustrated in Fig. 1 B, D, F. Mean values for single-channel conductance and reversal potentials are given in Table 1. In addition to the main-state, smaller and larger conductance states were found. At least two subconductance levels and one supraconductance level were observed. I–V relationships for the main-state activated by all agonists were linear with a reversal potential around 0 mV. The sub- and supraconductance levels also had linear I–V relationship with reversal potentials identical to the main-state. Subconductance states had single-channel conductances around 13 pS and 18 pS while the supraconductance level was around 31 pS for all agonists.

In order to support the suggestion that these additional levels are an inherent part of the GABAA receptor complex, channel substates should interconvert with the channel mainstate with direct transitions from one conductance level to another being observed. The data in Fig. 2 show that transitions between the main-state and the other conductance levels were observed with all agonists. Furthermore, the sub- and supraconductance levels were not present in the absence of main-state activity. Example of GABA-gated activity are shown in Fig. 2 A. The series of openings indicated by the arrowhead in the upper trace is shown on a faster time scale in the lower trace. The closed arrow points to a transition from the main-state to the 18-pS subconductance level while the open arrow shows an opening to the subconductance state. Similar transitions are shown for muscimol (Fig. 2A) and isoguvacine (Fig. 2C). The occurrence of transitions to different conductance levels is suggestive that these conductance levels are due to activation of one macromolecular complex and do not reflect the activity of two independent channels.

In order to look in more detail at the openings gated by GABA and its agonists outside-out patches were exposed to the various agonists for 2–10 min at a single holding potential (between −60 mV and −110 mV). Amplitude histograms for most patches showed two distinct peaks, one at the closed level and one at the main-state open level. Openings of the GABA-gated chloride channel to the sub- and supraconductance levels were rare, representing, at most, a few percent of the total number of openings in the majority of patches (eight of ten). In the remaining two outside-out patches, openings of the GABA channel to the 13-pS subconductance level...