

Ionotropic GABA Receptor From Lobster Olfactory Projection Neurons

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Zhainazarov, A. B., M. Wachowiak, A. Boettcher, S. Elenes, and B. W. Ache. Ionotropic GABA receptor from lobster olfactory projection neurons. *J. Neurophysiol.* 77: 2235–2251, 1997. This study reports an ionotropic GABA (γ -aminobutyric acid) receptor in projection neurons acutely dissociated from the olfactory lobe of the brain of the spiny lobster and analyzed by whole cell and cell-free patch-clamp recording. GABA evokes a macroscopic current in the cells that is linear from -100 to $+100$ mV, reverses at the imposed chloride equilibrium potential, has a permeability sequence of $\text{Cl}^- > \text{acetate} > \text{bicarbonate} > \text{phosphate} > \text{propionate}$ and $\text{SCN}^- > \text{Br}^- > \text{I}^- > \text{Cl}^- > \text{F}^-$, and is reversibly blocked by the Cl channel blocker picrotoxin but not *tert*-butylbicyclophosphorothionate (TBPS). The current is bicuculline insensitive and activated by muscimol, isoguvacine, *cis*-4-aminocrotonic acid (CACA), and *trans*-aminocrotonic acid (TACA), as well as by the GABA_C-receptor antagonists 4,5,6,7-tetrahydroisoxazolo [5,4,-c]pyridin-3-ol (THIP), 3-amino-1-propanesulfonic acid (3-APS), and imidazole-4-acetic acid (I-4AA), but not the GABA_B-receptor agonists baclofen and 3-aminopropylphosphonic acid (3-APA). Agonist potency for the receptor is TACA > muscimol > GABA > I-4AA > isoguvacine > 3-APS > CACA > THIP. Unitary chloride currents in cell-free, outside-out patches from the cells share enough of these pharmacological properties to indicate that the channel underlies the macroscopic current. The receptor mediates an inhibitory current in the cells in vivo. The receptor is similar, if not identical, to one from neurons cultured from the thoracic ganglia of the clawed lobster. The more extensive pharmacological characterization of the receptor reported here indicates that this lobster CNS receptor is pharmacologically distinct from previously characterized ionotropic GABA receptors.

INTRODUCTION

Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter that serves acts both pre- and post-synaptically either by directly gating a chloride channel or by modulating K^+ - or Ca^{2+} -selective channels via a G-protein linked second messenger cascade (review: Mody et al. 1994). Major classes of vertebrate GABA receptors are the metabotropic GABA_B and the ionotropic GABA_A and GABA_C receptors. Small differences in molecular structure that confer distinct functional properties on the receptor provide additional diversity within these three classes.

Multiple types of GABA receptors also occur in invertebrates, particularly in the arthropods where they have been studied in more detail. Although they do not clearly conform to the vertebrate classification, arthropod GABA receptors can directly gate chloride channels (reviews: Lunt 1991; Walker and Holden-Dye 1989) or can be associated with G proteins or K^+ channels (Bai and Sattelle 1995; Marder and

Paupardin-Tritsch 1978; Miwa et al. 1990). There is also additional diversity within these two major types. Pharmacologically distinct GABA receptors, for example, can occur in muscle and nerve of the same species of insect (Sattelle et al. 1988) and even in different muscles in the same species of crustacean (Dudel and Hatt 1976).

Although GABA was first identified as an inhibitory transmitter at the lobster neuromuscular junction and several types of GABA receptors have been identified and functionally characterized in the crustacean peripheral nervous system (Lunt 1991; Nistri and Constanti 1979), relatively little is known about crustacean GABA receptors in the central nervous system. GABA receptors in the CNS-associated stomatogastric and cardiac ganglia, like vertebrate GABA_A and insect GABA receptors, are activated by muscimol and blocked by picrotoxin (Cazalets et al. 1987; Kerrison and Freschi 1992). Jackel et al. (1994a,b) characterized an ionotropic GABA receptor of unknown function from cultured lobster thoracic ganglion neurons that, in addition to being activated by muscimol and blocked by picrotoxin, was activated by the specific vertebrate GABA_C receptor agonist *cis*-4-aminocrotonic acid (CACA). The latter finding suggested that this arthropod receptor may be of the vertebrate GABA_C type. Here, we characterize the pharmacological properties of a similar GABA receptor, if not identical to the one described by Jackel et al. that is expressed by olfactory interneurons in the brain (supraesophageal ganglion) of another lobster and mediates inhibition at the first relay in the olfactory pathway. The lobster CNS receptor appears to be pharmacologically distinct from other ionotropic GABA receptors described so far in vertebrates and other arthropods. Parts of this work were presented previously in abstract form (Wachowiak et al. 1995).

METHODS

Animals and preparations

Adult specimens of the Caribbean spiny lobster, *Panulirus argus*, were collected in the Florida Keys and maintained in the laboratory in running seawater on a diet of fish, squid, and shrimp. Two different preparations were used to record from the somata of projection neurons in the olfactory midbrain (deutocerebrum): acutely dissociated somata and cultured neurons.

ACUTELY DISSOCIATED SOMATA. Dissociated somata of the projection neurons were obtained by chilling lobsters on ice, removing the brain, and exposing the lateral soma clusters (cluster No. 10) (Sandeman et al. 1994) that exclusively contain the somata of the olfactory projection neurons. In some experiments, small

clumps of neurons were removed from the lateral cluster and placed in a flow-through chamber (1–2 ml) on the stage of an upright microscope (Axioskop, Carl Zeiss; $\times 40$ water-immersion objective) and superfused with *Panulirus* saline (PS; see *Solutions*) at 0.5 ml/min. In other experiments, the entire lateral cluster was removed from the brain and incubated for 10 min at 80 revs/min on an orbital shaker in 10 ml PS containing 2.5 mg papain and 12 mg L-cysteine. Clusters then were rinsed thoroughly with enzyme-free PS and cut into small pieces. These pieces were placed in 1.5 ml PS and triturated through a Pasteur pipette fire-polished to an inner tip diameter of 0.5 mm. The fully dissociated neurons were plated on glass cover slips and stored in PS at 4°C until used within 4 h. Cover slips were transferred to a recording chamber (0.3 ml) mounted on the stage of an inverted microscope (Axiovert 100, Carl Zeiss) and viewed with $\times 32$ phase-contrast optics.

CULTURED NEURONS. The somata of the projection neurons also were placed in primary culture as described previously for lobster olfactory receptor neurons (Fadool et al. 1991). Briefly, lateral soma clusters were removed from the brain and incubated for 15 min in 10 ml of 0.2- μ m filter sterilized PS containing papain (2.5 mg), L-cysteine (12 mg), penicillin (1%), streptomycin sulfate (1%), and amphotericin (1%). Proteolytic digestion was stopped by replacing the enzyme-containing solution with low-glucose L-15 culture medium supplemented with L-glutamine, dextrose, fetal calf serum, and BME vitamins. Clusters were then triturated and plated on poly-D-lysine-coated glass coverslips. Cells were maintained at saturated humidity at 24°C and used within 1–4 days.

Recording, data analysis, and stimulus delivery

INTEGRAL CURRENTS. Conventional whole cell voltage- and current-clamp recordings were obtained from the somata (Hamill et al. 1981). Perforated whole cell recordings were made as described by Horn and Marty (1988). In some experiments, gramicidin was used to perforate the membrane (Zhainazarov and Ache 1995). Patch pipettes were pulled from borosilicate glass (2-000-100, Drummond Scientific) to a tip diameter of $\sim 1 \mu\text{m}$ (bubble number = 4.5–5). Electrodes were coated with silicone elastomer (Sylgard, Dow-Corning) to reduce capacitance and fire-polished immediately before use. The pipettes had resistances of 4–9 M Ω when filled with patch solutions (see *Solutions*) and formed seals with resistances of 1–8 G Ω . The series resistance in conventional whole cell experiments was $< 10 \text{ M}\Omega$. Perforated whole cell recordings were started when the series resistance was $< 25 \text{ M}\Omega$. Signals were amplified using a commercial patch-clamp amplifier (Axopatch 200A, Axon Instruments, or Dagan 3900A, Dagan Corporation), low-pass filtered at 1 kHz (-3 dB , 4-pole Bessel filter). Most experiments were directly digitized at 2–5 kHz to the hard disk of an IBM-compatible computer using a TL-1 DMA with pClamp software (Axon Instruments). Some experiments were stored on videotape and analyzed on playback using Axotape software (Axon Instruments).

GABA and related compounds were pressure ejected onto single cells from glass micropipettes $\sim 2 \mu\text{m}$ diam using controlled pressure pulses (Picospritzer, General Valve; 20 ms to 1 s, 100 kPa; 0.5 pulse/min). The micropipette tip was brought to within 10–20 mm of the soma under visual control. Fast Green (1%), which had no obvious effect when tested by itself, was included in the pipette to visually confirm that the stimulus reached the soma. The concentration reported is the pipette concentration, with no attempt to correct for dilution. In some cases a six-barrel pipette was used to apply multiple stimuli to the same cell at 2-min intervals. GABA antagonists were studied by adding them to the bath before pressure ejecting GABA (plus the concentration of antagonist in the bath) on the cell.

UNITARY CURRENTS. For single-channel recording, patch pipettes were fabricated from borosilicate filament glass (1.50 mm OD; 0.86 mm ID; Sutter Instrument) and fire-polished to a tip diameter $\sim 1 \mu\text{m}$ (bubble number, 4.5). The pipettes were coated with silicone elastomer. The patch pipettes had resistances of 8–10 M Ω when filled with patch pipette solutions (see below for composition) and formed seals of 10–20 G Ω . Single-channel currents were recorded with an Axopatch-200A amplifier, low-pass filtered at 1 kHz (-3 dB ; 4-pole Bessel filter), and digitized at 10 kHz by an IBM-compatible computer with TL-1 DMA interface and pClamp software. Statistical analyses of single-channel current traces also were done with pClamp software. All potentials were corrected for the liquid junction potentials at the pipette tip and at the indifferent electrode as described by Neher (1992).

Patches excised in the outside-out configuration were exposed to GABA and related compounds by moving the tip of the pipette in front of one tube of a nine-channel rotatory “sewer pipe” perfusion system (RSC-100, Biologic) placed in the culture dish. PS continuously flowed from the tube (100 mm ID) in a laminar pattern and completely engulfed the patch. The remaining identical eight tubes either contained PS or drug-containing PS appropriate to the particular experiment so as to create nine parallel, defined streams. Electronically rotating one of the tubes in front of the patch pipette allowed a complete change of background solution within 10 ms. Unless noted otherwise, results are expressed as the sample means \pm SD of n observations. All experiments were carried out at room temperature (20–22°C).

Solutions

PS consisted of (in mM) 460 NaCl, 13 KCl, 13 CaCl₂, 10 MgCl₂, 14 Na₂SO₄, 1.7 glucose, and 3 *N*-2-hydroxyethyl piperazine-*N'*-2 ethane sulfonic acid (HEPES), at pH 7.4 adjusted with NaOH. Standard K-acetate patch solution consisted of (in mM) 30 NaCl, 11 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 HEPES, 1 CaCl₂, 180 K-acetate, 5 Na-ATP, 5 MgCl₂, and 522 glucose, at a pH of 7.2. KCl patch solution consisted of (in mM) 210 KCl, 1 CaCl₂, 11 EGTA, 10 HEPES, 5 Na-ATP, 5 MgCl₂, and 522 glucose, at a pH of 7.2. In some experiments, part of the KCl in the KCl patch solution was substituted by an equivalent concentration of Na-isethionate, as described in the text and figure legends. Low-chloride Cs acetate patch solution consisted of (in mM) 180 Cs acetate, 30 tetraethylammonium chloride (TEACl), 11 EGTA, 1 CaCl₂, 10 HEPES, 5 Na-ATP, 5 MgCl₂, and 522 glucose, at a pH of 7.2. High-chloride Cs patch solution consisted of (in mM) 180 CsCl, 30 TEACl, 11 EGTA, 1 CaCl₂, 10 HEPES, 5 Na-ATP, 5 MgCl₂, and 522 glucose, at a pH of 7.2. The pH of all patch pipette solutions was adjusted with 1 M Tris(hydroxymethyl) amino-methane (TRIS) base. In experiments performed to measure permeabilities of inorganic and organic anions through the channel, the patch pipette solution consisted of (in mM) 10 HEPES, 11 EGTA, 1 CaCl₂, 5 Na-ATP, 5 MgCl₂, 522 glucose, and 210 potassium salt of one of the following anions: SCN⁻, I⁻, Br⁻, F⁻, Cl⁻, bicarbonate, acetate, phosphate, and propionate, at a pH 7.2 adjusted with TRIS base and at $\sim 1,000$ mmol/kg osmolarity adjusted with glucose.

GABA and related compounds were dissolved in PS, frozen in 100- μ l aliquots, and diluted 10-fold immediately before use. Antagonists were made fresh daily by dissolving in PS. Stock solutions of (+)-hydrastine and *tert*-butylbicyclophosphorothionate (TBPS) were dissolved in ethanol and diluted 100- to 1,000-fold in PS before use. Gramicidin stock solution consisted of 10 mg/ml of the antibiotic in dimethyl sulfoxide prepared by sonicating for 5 min and stored at -20°C until use. The stock solution was made fresh every third day. The stock solution was diluted for use to 200 $\mu\text{g}/\text{ml}$ in KCl patch pipette solution and sonicated for 10 min before loading the pipettes. All inorganic salts were

purchased from Fisher Scientific. All organic chemicals and drugs were obtained from Sigma (St. Louis, MO) or from RBI (Natick, MA) except for HEPES, which was obtained from Research Organics.

RESULTS

GABA-induced macroscopic current

GABA (100 μ M) elicited a large macroscopic current at -60 mV in acutely dissociated projection neurons (Fig. 1A) that was associated with an increase in membrane conductance (Fig. 1B). The peak amplitude of the GABA-induced current at a holding potential of -70 mV varied from 72 to 775 pA ($n = 9$). The GABA-induced current was relatively stable in the conventional whole cell recording configuration and decreased $<30\%$ of its initial value during 20 min. The current showed little, if any, desensitization; continuous exposure to 500 μ M GABA reduced the magnitude of the outward current $<12 \pm 4\%$ of its initial amplitude during 5 s ($n = 3$, data not shown). The current was fast, being activated with a latency of 22 ± 2 ms ($n = 6$) and reaching half-maximal amplitude within 22 ± 8 ms ($n = 6$). In one cell where the spritzing pipette was $3\text{--}5$ μ m from the soma, GABA evoked an outward current within 10 ms that peaked in the next 11 ms, suggesting that the current was mediated by an ionotropic GABA receptor.

The amplitude of the GABA-induced current in cultured neurons was dose dependent from 1 to 1 mM (Fig. 1C). Normalizing the peak current to the response to 1 mM GABA at a holding potential of -10 mV generated a dose response curve (solid line, Fig. 1D) that could be fitted with the equation

$$I/I_{\max} = [C]^n / ([C]^n + EC_{50}^n) \quad (1)$$

where $[C]$ is the agonist concentration, I is the normalized current evoked by a given concentration of agonist, I_{\max} is the maximal agonist-induced current, EC_{50} is the half-effect concentration, and n is the Hill coefficient. Using this equation, the EC_{50} was 33.5 ± 0.9 mM (mean \pm SE), with a Hill coefficient of 1.5 ± 0.1 (SE).

Current-voltage relationship and ion selectivity of the GABA-induced macroscopic current

The polarity of the GABA-induced current in the acutely dissociated somata, measured with K-acetate patch solution (180 mM K-acetate, 30 mM NaCl) in the pipette, was outward at -40 mV, but became inward near -50 mV (Fig. 2A). The current-voltage (I - V) relationship for all cells studied was essentially linear (Fig. 2B). With KCl patch solution (210 mM KCl) in the pipette to give a calculated equilibrium potential for Cl^- (E_{Cl}) of -22.9 mV, the GABA-induced current reversed polarity at -24.1 ± 3.0 mV (SE; $n = 5$, Fig. 3A). With high-chloride Cs patch solution (180 mM CsCl and 30 mM TEACl) in the pipette, the GABA-induced current continued to reverse near the calculated E_{Cl} of -22.9 mV [-23.3 ± 3.4 mV (SE); $n = 5$, data not shown]. The reversal potential of the GABA-evoked current corresponded strictly to the chloride equilibrium potential

calculated from the Nernst equation (Fig. 2C). These data suggest that the GABA-evoked current is carried by Cl^- .

The permeability sequence of the GABA-channel was determined by substituting all of the KCl (210 mM) in the KCl pipette solution with the equivalent concentration of KX , where X^- is the anion of interest. The GABA-evoked current reversed polarity at $+31.3 \pm 2.5$ (SE; $n = 3$) for SCN^- , $+12.8 \pm 0.9$ (SE; $n = 10$) for I^- , $+22.2 \pm 2.5$ (SE; $n = 3$) for Br^- , -83.6 ± 3.1 (SE; $n = 8$) for F^- , -49.4 ± 1.8 (SE; $n = 9$) for acetate, -58.7 ± 2.0 (SE; $n = 10$) for bicarbonate, -67.3 ± 2.6 (SE; $n = 3$) for phosphate, and -75.8 ± 2.4 (SE; $n = 10$) for propionate (Fig. 3). The permeability of these anions relative to that of Cl^- (P_X/P_{Cl}) was calculated from the reversal potential (E_{rev}) by using the following equation

$$P_X/P_{Cl} = ([Cl^-]_o/[X^-]_i) \exp(FE_{rev}/RT) \quad (2)$$

where R is the gas constant, F the Faraday constant, T the absolute temperature, $[Cl^-]_o$ the extracellular concentration of Cl^- , $[X^-]_i$ the intracellular concentration of anion of interest. The permeability ratio sequences relative to Cl^- (P_X/P_{Cl}) were Cl^- (1.00) $>$ acetate (0.35) $>$ bicarbonate (0.24) $>$ phosphate (0.17) $>$ propionate (0.12) for large polyatomic anions and SCN^- (8.51) $>$ Br^- (5.95) $>$ I^- (4.10) $>$ Cl^- (1.00) $>$ F^- (0.09) for small anions, respectively.

Pharmacological properties of the GABA-induced macroscopic current

ANTAGONISTS. Picrotoxin (PTX) significantly reduced the current elicited by pressure ejection 100 μ M GABA on acutely dissociated somata (see Table 1). PTX (100 μ M) reversibly reduced the response to 100 μ M GABA by 81.9% (Fig. 4A, top). The GABA-evoked current was inhibited by PTX (100 μ M) in equal extent at all tested membrane potentials (-100 to $+100$ mV), indicating that the PTX-inhibition is voltage independent (Fig. 4B). The effect of picrotoxin was dose-dependent (Fig. 4C, ●). The dose-response relationship could be fitted by the following equation (Fig. 4C, —●—)

$$I/I_{\max} = 1 - \{[C]^n / ([C]^n + IC_{50}^n)\} \quad (3)$$

where $[C]$ is the concentration of antagonist, IC_{50} the half-effect concentration of antagonist [18.5 ± 2.7 μ M (SE)], n the Hill coefficient [0.97 ± 0.13 (SE)], I and I_{\max} the GABA-evoked current in the absence and in the presence of antagonist, respectively.

TBPS, a potent ligand for the PTX binding site in vertebrate GABA_A receptors, at 100 μ M concentration reversibly inhibited the GABA-evoked current (GABA, 100 μ M) by only 5.7% (Fig. 4A, middle). Though the effect of TBPS was dose dependent (Fig. 4C, ○; 1–800 μ M of TBPS), even 0.8 mM TBPS inhibited the GABA-evoked current (GABA, 100 μ M) by only 36.3% ($n = 5$). The fit of the Eq. 3 to the data yielded the following parameters for TBPS: $IC_{50} = 1.32 \pm 0.16$ mM (SE) and $n = 1.2 \pm 0.2$ (SE). This relative insensitivity to TBPS also characterizes GABA-evoked currents in fish horizontal cells (Qian and Dowling 1994).

Bicuculline methiodide, the water-soluble form of bicu-

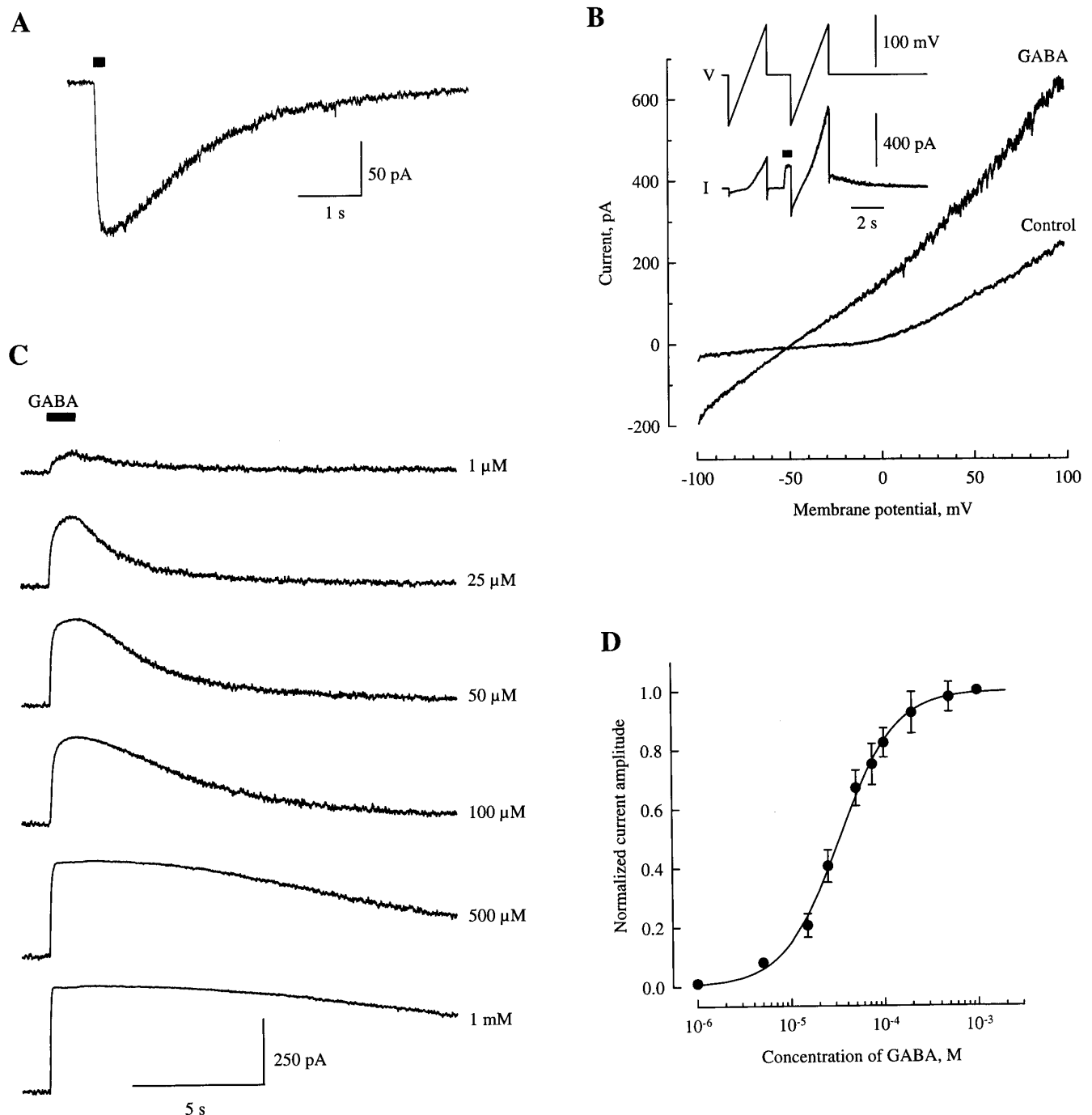
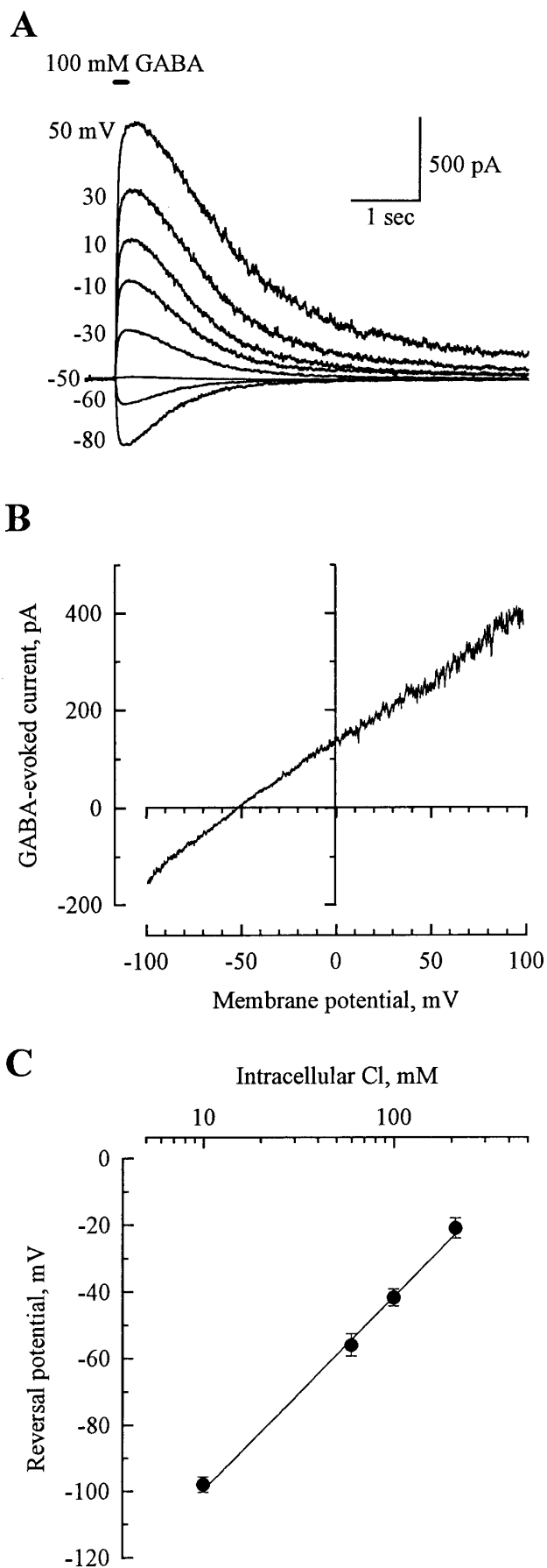


FIG. 1. γ -aminobutyric acid (GABA)-evoked responses. *A*: pressure ejecting $100\ \mu\text{M}$ GABA (bar) onto an acutely dissociated soma at a holding potential of $-60\ \text{mV}$ elicits a large transient current. *B*: GABA-evoked current is associated with an increase in membrane conductance. Current-voltage relationships of whole cell currents measured before (control) and during (GABA) GABA-response. *Inset*: experimental protocol shown. Voltage ramps (-100 to $100\ \text{mV}$; $83.3\ \text{mV/s}$) were applied before and during GABA response. *Top* (V) and *bottom* (I) traces indicate membrane potential and current, respectively. Holding potential, $-60\ \text{mV}$. Timing of GABA pulse ($100\ \mu\text{M}$, $200\ \text{ms}$) is depicted above current trace by solid bar. *C*: records from 1 neuron evoked by concentration of GABA (bar) indicated near each trace. Holding potential, $-10\ \text{mV}$. *D*: plot of dose-response relationship of GABA-induced current as measured in cultured olfactory projection neurons. Ordinate, peak amplitude of currents induced by GABA at each concentration, normalized to current induced by $1\ \text{mM}$ GABA. Each point is average of 5–7 cells. Error bars indicate standard deviation. Solid line is fit of Eq. 1 (see text) to data. For A–C, standard K-acetate patch solution was used in the pipette.

culline, (Fig. 4A, *bottom*; $n = 5$) and (+)- β -hydrastine ($n = 5$; data not shown), both competitive antagonists of vertebrate GABA_A receptors, had no effect on the GABA-

induced current up to concentrations of $1\ \text{mM}$. The vertebrate glycine receptor antagonist strychnine ($100\ \mu\text{M}$) was also without effect. Three antagonists of a histamine (HA)-in-



duced chloride current expressed on at least some of these cells (Wachowiak and Ache 1995)—cimetidine, ranitidine, and D-tubocurarine—also had no effect on the response to GABA, indicating that GABA activates a distinct receptor from that activated by HA.

AGONISTS. GABA receptor agonists were identified by their ability to elicit a current that reversed near E_{Cl} and that could be blocked by 100 μ M PTX in paired applications with GABA. The GABA_B agonists baclofen (200 μ M; $n = 3$, data not shown) (Hill and Bowery 1981) and 3-aminopropylphosphonic acid (3-APA; 0.1–1.0 mM; Fig. 7A, $n = 10$) (Slaughter and Pan 1992) failed to elicit a current when pressure ejected on to the acutely dissociated somata. 3-APA (100 μ M), which also antagonizes GABA_C receptors (Pan and Lipton 1995), did not show any effect on the GABA-evoked current ($n = 3$, data not shown). The GABA_A-receptor agonists, muscimol (100 μ M) and isoguvacine (100 μ M) (Barker and Mathers 1981), however, elicited PTX-sensitive currents in paired applications with GABA (Fig. 5A). The muscimol- and isoguvacine-evoked currents were both PTX sensitive (Fig. 5B, data shown only for muscimol). With KCl patch pipette solution, the currents activated by both agonists reversed close to the predicted E_{Cl} of -22.9 mV: at -21.8 ± 1.7 mV (SE; $n = 4$) for muscimol and at -24.6 ± 1.9 mV (SE; $n = 4$) for isoguvacine, respectively (Fig. 5C). For five cells, the current elicited by muscimol (100 μ M) and isoguvacine (100 μ M) at -60 mV was $95.2 \pm 10.7\%$ (SD) and $37.3 \pm 14.2\%$ (SD) of the GABA-evoked current (GABA, 100 μ M) on the same cell (Fig. 5A).

CACA (0.1–1 mM), a conformationally restricted GABA analogue that is an agonist for the vertebrate GABA_C receptor (Feigenspan et al. 1993; Qian and Dowling 1993) and for an ionotropic GABA-receptor identified in neurons cultured from the clawed lobster *Homarus gammarus* (Jackel et al. 1994b), also elicited a PTX-sensitive current (Fig. 5, A and B). The CACA-evoked current reversed near E_{Cl} in KCl patch solution [-23.7 ± 2.2 mV (SE); $n = 3$; Fig. 5C]. Another agonist of GABA_C-receptor, *trans*-4-aminocrotonic acid (TACA; 100 μ M) (Feigenspan et al. 1993; but also see Woodward et al. 1993), evoked a PTX-sensitive current (data not shown, $n = 3$) and reversed at -23.1 ± 3.1 (SE; $n = 3$) in KCl patch solution ($E_{Cl} = -22.9$ mV). In paired applications 100 μ M CACA- and 100 μ M TACA-evoked currents were $7.8 \pm 3.9\%$ (SD; $n = 5$) and $96.2 \pm 8.8\%$ (SE; $n = 5$) of the GABA-evoked current (GABA, 100 μ M) on the same cell at -60 mV ($n = 5$; Fig. 5A).

3-APS (100 μ M), an antagonist of GABA_C-receptor

FIG. 2. Current-voltage relationship and Cl^- specificity of GABA-induced current. **A**: currents evoked by pressure ejecting 100 μ M GABA (bar) onto an acutely dissociated soma at holding potentials indicated near each trace. **B**: plot of current-voltage relationship of GABA-induced current, which reversed at -51.9 mV. GABA (100 μ M) pulse, 1 s. Pipette, standard K-acetate patch solution. **C**: plot of shifts in reversal potential of GABA-evoked currents when various amounts of KCl in the KCl patch solution (see METHODS) were substituted by equivalent concentrations of Na-isethionate. Bath solution, *Panulirus* saline (PS). GABA (100 μ M) pulse, 1 s. Current-voltage relationship was measured by voltage ramps (see Fig. 1). Points shown are means \pm SE of 4–5 different experiments. Solid lines were calculated from Nernst equation for Cl^- .

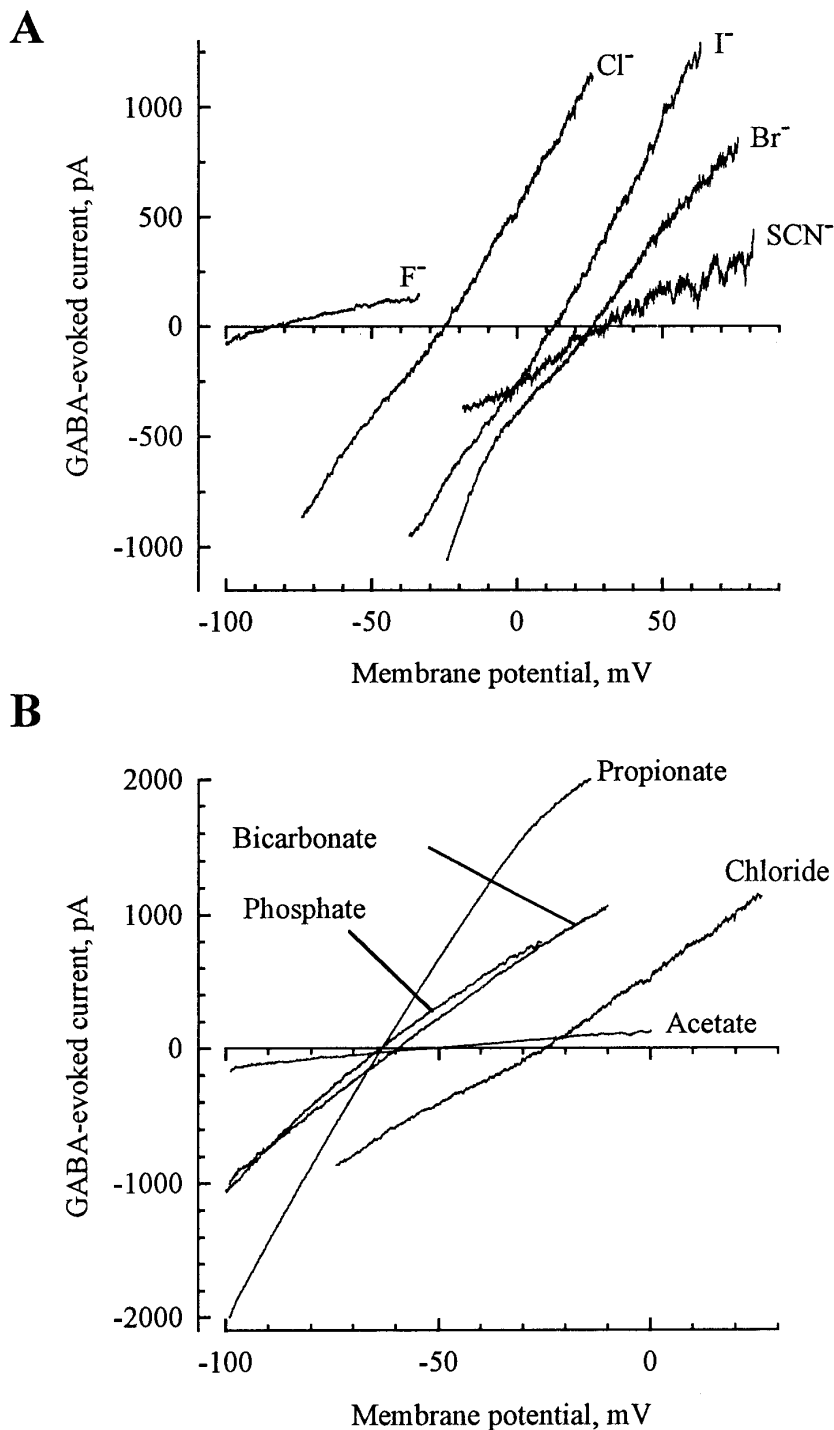


FIG. 3. Effect of different intracellular small (A) and large (B) anions on reversal potential of GABA-evoked whole cell current. All KCl (210 mM) in the KCl pipette solution (see METHODS) was substituted by equivalent concentration of the following salts (reversal potential \pm SE in millivolts; $n = 3 \pm 10$): KSCN ($+31.3 \pm 2.5$), KI ($+12.8 \pm 0.9$), KBr ($+22.2 \pm 2.5$), KF (-83.6 ± 3.1), K-bicarbonate (-58.7 ± 2.0), K-acetate (-49.4 ± 1.8), K-phosphate (-67.3 ± 2.6), and K-propionate (-75.8 ± 2.4). Bath solution, PS. GABA ($100 \mu M$) pulse, 1 s. The current-voltage relationships were measured by voltage ramps (see Fig. 1).

(Woodward et al. 1993), elicited an inward current at -60 mV in paired applications with GABA ($100 \mu M$; Fig. 5A). The 3-APS-evoked current was PTX-sensitive ($n = 3$, data not shown) and reversed at -21.7 ± 4.2 mV (SE; $n = 5$) in KCl patch solution. In paired applications, the currents evoked by $100 \mu M$ 3-APS was $19.8 \pm 7.5\%$ (SD; $n = 5$) of the GABA-evoked current (GABA, $100 \mu M$) on the same cell at -60 mV (Fig. 5A).

To determine whether GABA, muscimol, isoguvacine, 3-APS, TACA, and CACA act on the same receptor, both GABA and one of four GABA-receptor agonists were

pressure ejected onto the cell at 0.5-s intervals. Applying 1 mM GABA (100 ms) followed by 1 mM muscimol (100 ms) evoked a current of $-1,014$ pA (Fig. 6A, top; representative trace of 5 cells). Applying 1 mM muscimol (100 ms) followed by 1 mM GABA (100 ms) evoked a current of $-1,019$ pA (Fig. 6A, middle top). These results indicate that the GABA- and muscimol-evoked currents are not additive. GABA (1 mM) and CACA (1 mM) gave similar results; GABA/CACA, -910 pA; CACA/GABA, -900 pA ($n = 5$, Fig. 6A, middle-bottom and bottom). Similar nonadditivity occurred for the pairs GABA/

TABLE 1. Effects of GABA-receptor agonists and antagonists on projection neurons in the lobster olfactory lobe

Compound	Concentration	Current, %	Inhibition, %	n
GABA _A -receptor agonists				
GABA	1 mM	100		
Muscimol ^a	1 mM	92.9 ± 8.6	5	5
Isoguvacine ^b	1 mM	90.1 ± 7.7		5
GABA _B -receptor agonists				
3-APA	1 mM	NE	11	10
Baclofen	1 mM	NE		3
GABA _C -receptor agonists				
TACA ^c	1 mM	96.5 ± 10.9		4
CACA ^a	1 mM	38.6 ± 12.0		4
GABA _A -receptor antagonists				
Bicuculline methiodide	1 mM		NE	5
(+)-β-hydrastine	1 mM		NE	5
GABA _C -receptor antagonists				
THIP ^d	1 mM	18.9 ± 9.1		4
I-4AA	1 mM	95.1 ± 5.1		4
3-APA ^e	1 mM		NE	3
3-APS	1 mM	91.5 ± 5.9		4
Cl ⁻ -channel blockers				
Picrotoxin	100 μM		85.0 ± 2.75	
TBPS	100 μM		5.9 ± 3.56	

Relative current and percentage of block were determined relative to GABA-evoked current in paired applications on the same cell. NE, no effect; 3-APA, 3-aminopropylphosphonic acid; TACA, *trans*-aminocrotonic acid; CACA, *cis*-4-aminocrotonic acid; THIP, 4,5,6,7-tetrahydroisoxazole [5,4-c]pyridin-3-ol; I-4AA, imidazole-4-acetic acid; 3-APS, 3-amino-1-propanesulfonic acid; TBPS, *tert*-butylbicyclophosphorothionate. Data are means ± SD. ^a Muscimol is also activates the GABA_C receptor (Qian and Dowling 1993). ^b Isoguvacine is also a GABA_C receptor antagonist (Qian and Dowling 1994). ^c Both TACA and CACA also activate the GABA_C receptor (Woodward et al. 1993). ^d THIP is also a potent GABA_A receptor agonist (Krogsgaard et al. 1977; Alger and Nicoll 1982). ^e 3-APA is also a GABA_B receptor agonist (Slaughter and Pan 1992).

TACA (TACA/GABA), GABA/3-APS (3-APS/GABA), and GABA/isoguvacine (isoguvacine/GABA, $n = 5$ each pair; data not shown). These results indicate that GABA, muscimol, isoguvacine, 3-APS, TACA, and CACA act on the same receptor.

The amplitude of the currents evoked by muscimol, isoguvacine, 3-APS, TACA, and CACA were dose dependent between 1 μM and 1 mM (Fig. 6C). The data could be fit by the Hill Eq. 1 with the following parameters: $EC_{50} = 28.6 \pm 1.2 \mu\text{M}$ (SE) and $n = 1.88 \pm 0.12$ (SE) for muscimol; $EC_{50} = 17.6 \pm 2.0 \mu\text{M}$ (SE) and $n = 1.85 \pm 0.15$ (SE) for TACA; $EC_{50} = 356.1 \pm 15.6 \mu\text{M}$ (SE) and $n = 2.99 \pm 0.38$ (SE) for CACA; $EC_{50} = 170.0 \pm 4.9 \mu\text{M}$ (SE) and $n = 2.86 \pm 0.18$ (SE) for isoguvacine; $EC_{50} = 285.2 \pm 14.3 \mu\text{M}$ (SE) and $n = 2.28 \pm 0.24$ (SE) for 3-APS. Of these six compounds, the agonist potency sequence, based on the comparison of the half-effect concentrations, was TACA > muscimol > GABA > isoguvacine > 3-APS > CACA. To determine whether some of these compounds were acting as partial agonists, we measured the current evoked by 1 mM of each agonist paired with 1 mM GABA on the same cell (Fig. 6B). The percentage of the agonist-evoked current relative to GABA-evoked current was $96.5 \pm 10.9\%$ (SD; $n = 4$) for TACA, $92.9 \pm 8.6\%$ (SD; $n = 5$) for muscimol, $38.6 \pm 12.0\%$ (SD; $n = 6$) for CACA, $91.5 \pm 5.9\%$ (SD; $n = 4$) for 3-APS, and $90.1 \pm 7.7\%$ (SD; $n = 4$) for isoguvacine, respectively. CACA (1 mM) activated only 38.6% of the maximal GABA-evoked current, suggesting that CACA is only a partial agonist.

Imidazole-4-acetic acid (I-4AA) (100 μM), a GABA_C receptor antagonist (Kusama et al. 1993; Qian and Dowling 1994), evoked an inward current at -60 mV in combination with 100 μM GABA that was $10.8 \pm 1.8\%$ (SE, $n = 4$)

of the GABA-evoked current (Fig. 7A). PTX (100 μM) reversibly blocked $82.5 \pm 1.9\%$ (SD, $n = 4$) of the I-4AA-evoked current (Fig. 7B, top). The I-4AA-evoked current reversed at -21.5 ± 2.4 mV (SE, $n = 3$) near E_{Cl} (Fig. 7C). 4,5,6,7-tetrahydroisoxazole [5,4-c]pyridin-3-ol (THIP, 100 μM), a GABA_A-receptor agonist (Alger and Nicoll 1982; Barker and Mathers 1981; Krogsgaard-Larsen et al. 1977) and GABA_C-receptor antagonist (Kusama et al. 1993; Qian and Dowling 1994; Woodward et al. 1993), also elicited an inward current at -60 mV, but one that was $30.6 \pm 12.7\%$ (SD, $n = 4$) of the 100 μM GABA-evoked current (Fig. 7A). The THIP-evoked current reversed at -23.6 ± 1.8 mV (SE, $n = 3$), near E_{Cl} (Fig. 7C), and was blocked by 100 μM PTX, but only $57.0 \pm 19.7\%$ (SD, $n = 5$; Fig. 7B, bottom). The reduced antagonist action of PTX for the THIP-evoked current compared with that of the other agonists may indicate that the type of agonist bound may influence the efficacy of PTX as a chloride channel blocker. GABA-, I-4AA-, and THIP-evoked currents measured in the same cell were not additive (Fig. 8A), suggesting that these compounds act on the same receptor. The dose-response relationships for I-4AA and THIP (Fig. 8C) could be fit to Eq. 1 with the following parameters: $EC_{50} = 419.3 \pm 1.6 \mu\text{M}$ (SE) and $n = 0.92 \pm 0.17$ (SE) for THIP; $EC_{50} = 77.6 \pm 1.9 \mu\text{M}$ (SE) and $n = 1.90 \pm 0.10$ (SE) for I-4AA. The THIP (1 mM)-evoked current was $18.9 \pm 9.1\%$ (SD, $n = 4$) and the I-4AA (1 mM)-evoked current was $95.1 \pm 5.1\%$ (SD, $n = 4$) of the 1 mM GABA-evoked current (Fig. 8B), indicating that THIP is only a partial agonist.

Overall, the agonist potency sequence ($K_{1/2}$, μM) was TACA (17.6) > muscimol (28.6) > GABA (33.5) > I-4AA (77.6) > isoguvacine (170.0) > 3-APS (285.2) > CACA (356.1) > THIP (419.3).

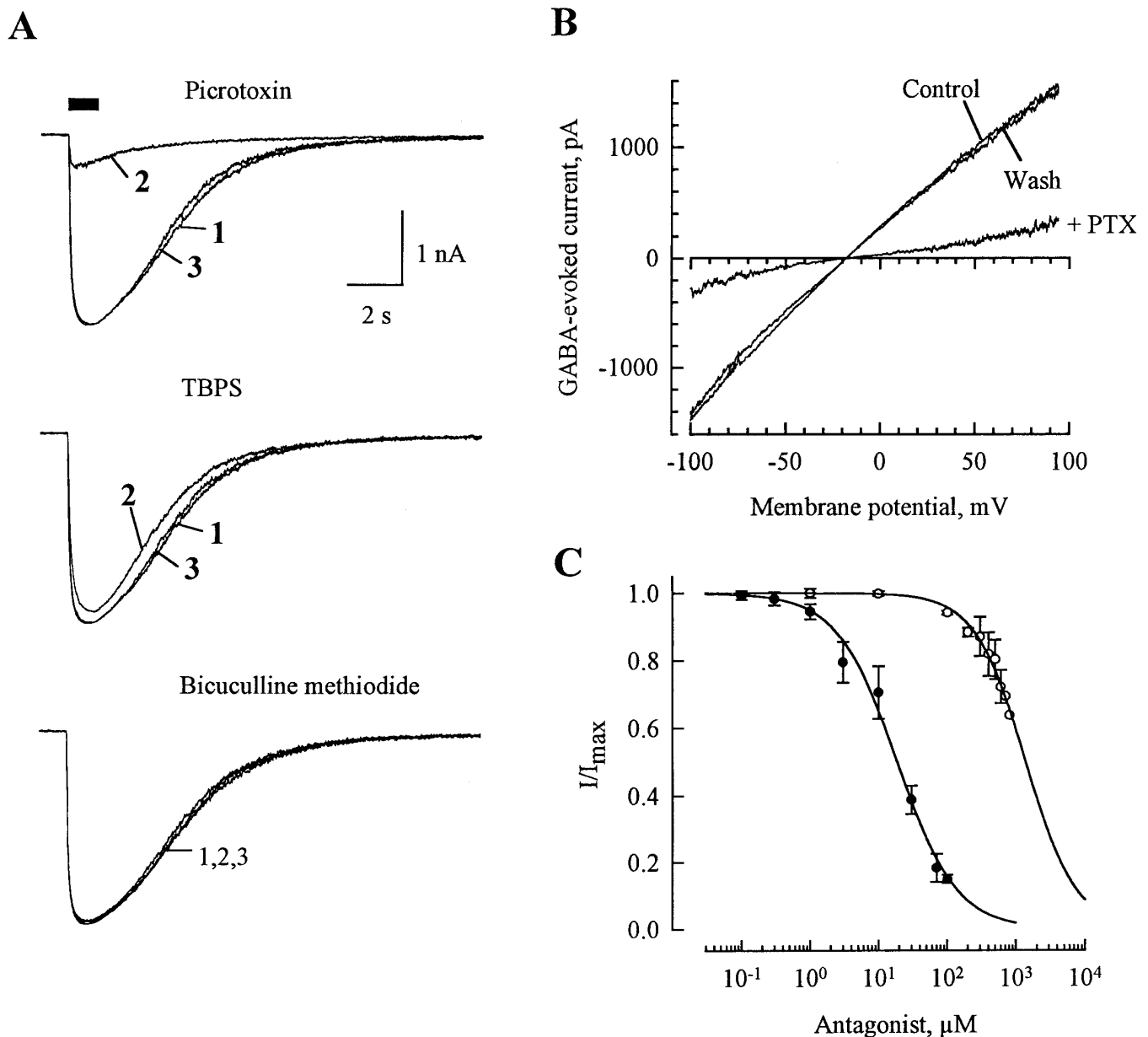


FIG. 4. A: effect of picrotoxin (PTX, 100 μM ; top), *tert*-butylbicyclophosphorothionate (TBPS, 100 μM ; middle), and bicuculline methiodide (100 μM ; bottom) on the GABA-evoked current. GABA, 100 μM . Timing of stimulus delivery is depicted by solid bar above current traces (see METHODS for details of stimulus delivery). Holding potential, -60 mV. Pipette, KCl patch solution. All 3 groups of traces were obtained from same cell. In each group of superimposed current traces control, effect of antagonist, and wash are marked by 1, 2, and 3, respectively. B: current-voltage relationship of GABA-evoked current for same cell in absence and in presence of 100 μM PTX; GABA, 100 μM . Current-voltage relationships were obtained by voltage ramps (see Fig. 1). C: concentration curve of effect of PTX (\bullet) and TBPS (\circ) on GABA-evoked current. Normalized amplitudes (I/I_{max}) of GABA-evoked current are plotted against of PTX and TBPS concentrations. I_{max} is GABA-evoked current in absence of antagonist. Solid lines are fit of Eq. 3 to data with following parameters: $IC_{50} = 18.5$ μM and $n = 0.97$ for PTX; $IC_{50} = 1.32$ mM and $n = 1.2$ for TBPS. GABA, 100 μM . Pipette, KCl patch solution. Holding potential, -60 mV. Points shown are means \pm SE of results of 4–7 different cells.

Unitary currents

GABA reversibly evoked unitary currents in outside-out patches taken from the soma of cultured olfactory projection neurons, indicating that GABA directly gates an ion channel ($n = 20$, Fig. 9A). The current-voltage relationship of the GABA-gated channel was linear between -70 and $+60$ mV

(Fig. 9, B–D), and the main open state had a slope conductance of 54.9 ± 0.7 pS (SE, $n = 6$).

PTX (100 μM) considerably decreased the unitary currents evoked by GABA (20 μM) without affecting the amplitude of the current ($n = 5$, Fig. 10A), suggesting that PTX exerts its antagonistic effect by reducing the channel open probability. The effect of PTX was fully reversible.

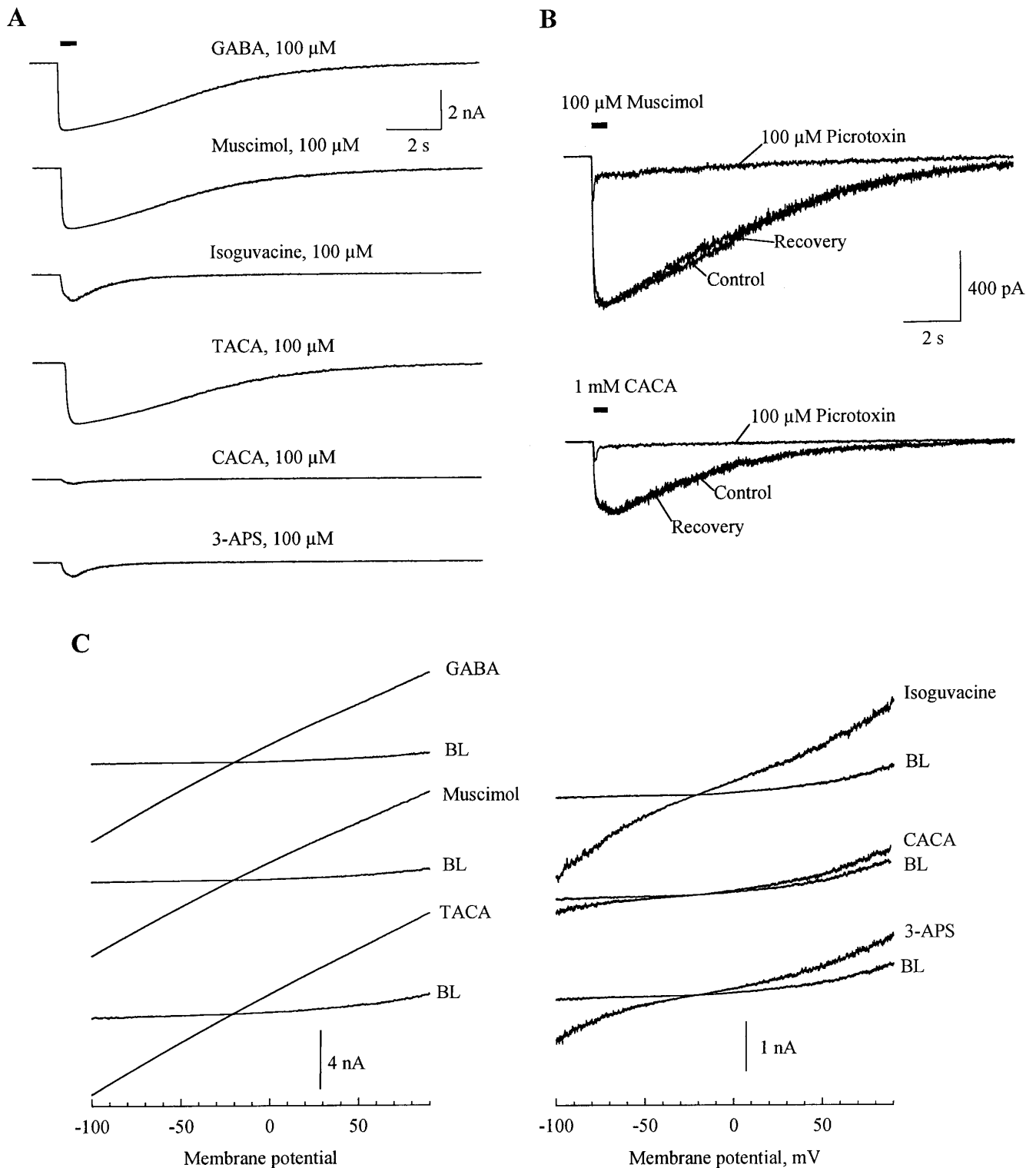


FIG. 5. *A*: responses of a projection neuron to GABA, muscimol, isoguvacine, *trans*-aminocrotonic acid (TACA), *cis*-4-aminocrotonic acid (CACA), and 3-amino-1-propanesulfonic acid (3-APS). Agonist, 100 μM . Holding potential, -60 mV. Pipette, KCl patch solution. All 6 current traces were obtained from same cell. *B*: voltage-clamp records from same projection neuron showing that 100 μM picrotoxin reversibly blocks current evoked by 100 μM muscimol (*top*) and 1 mM CACA (*bottom*). Pipette, high chloride Cs patch solution. Holding potential, -60 mV. Solid bars above current traces in *A* and *B* indicate timing of stimulus pulse. *C*: current-voltage relationships of whole cell currents measured before (BL) and during response evoked by 100 μM GABA or one of the following GABA analogues: muscimol, TACA, isoguvacine, CACA, and 3-APS. Value of reversal potential of GABA- (or GABA analogue)-evoked current is a potential where current-voltage curve of GABA- (or GABA analogue)-response intercepts leakage current-voltage curve (BL stands for baseline): -19.9 mV, GABA; -20.4 mV, muscimol; -20.8 mV, TACA; -20.9 mV, isoguvacine; -23.3 mV, CACA; -20.7 mV, 3-APS. Current-voltage relationships were obtained by voltage ramps (see Fig. 1). Pipette, KCl (210 mM) patch solution.

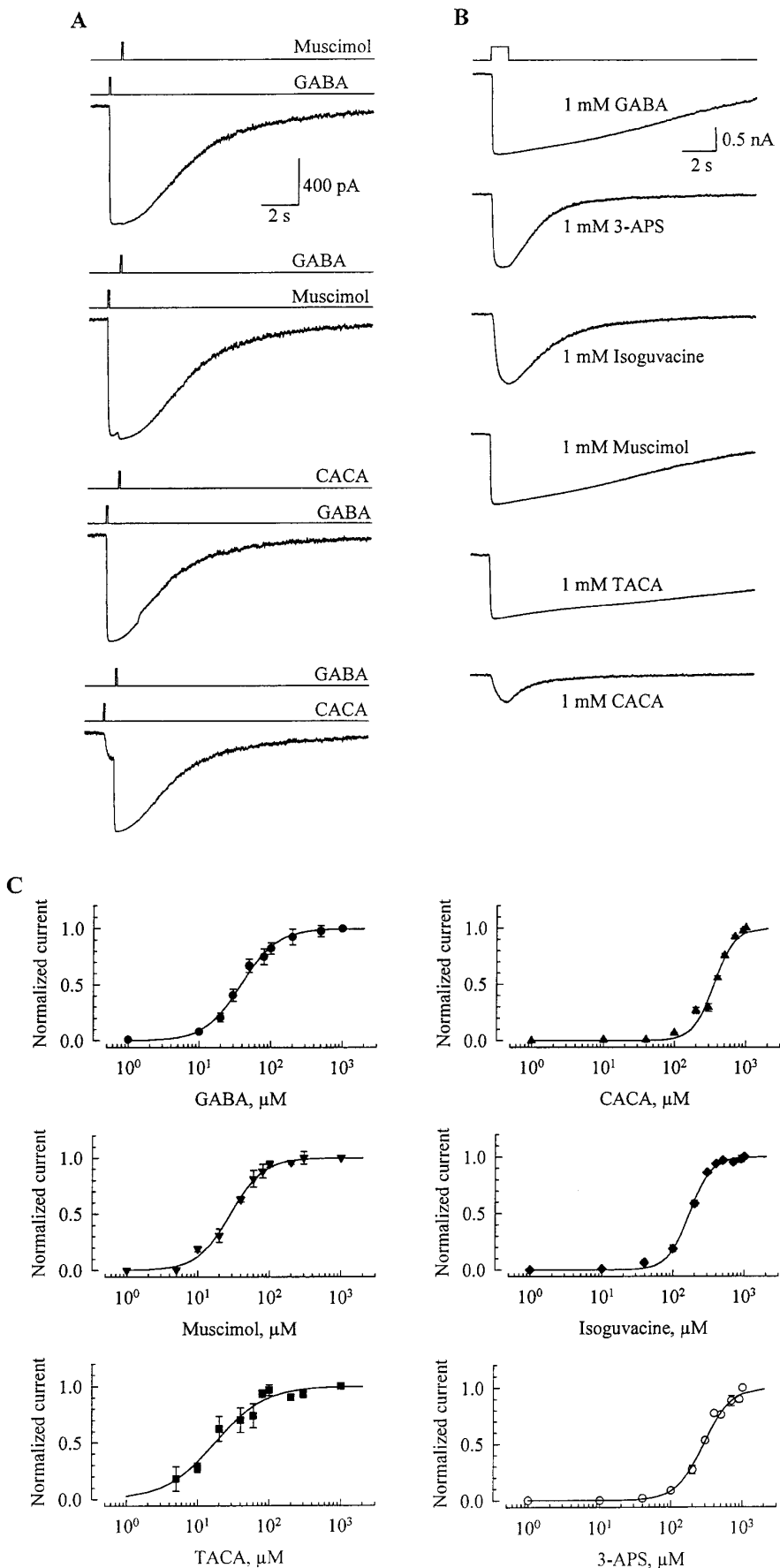
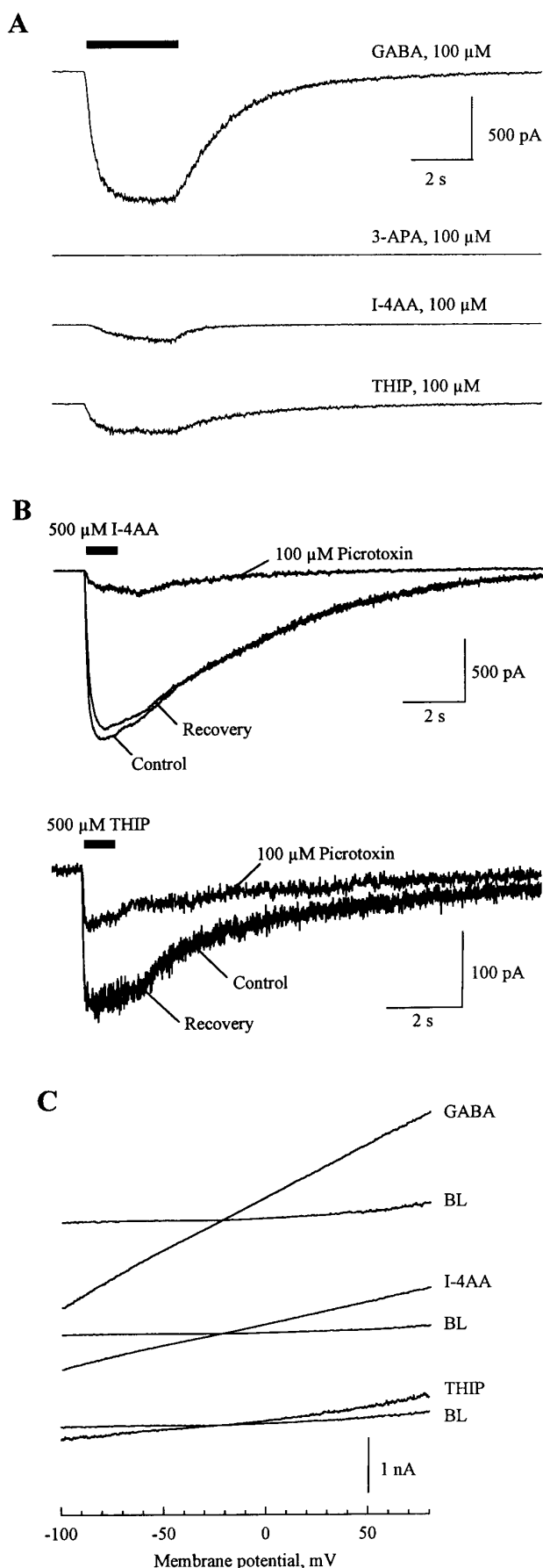


FIG. 6. *A*: responses evoked by successive application of 1 mM GABA and 1 mM muscimol (or CACA). Agonist type, sequence of application, and timing of stimulus are indicated above each current trace. Two *top traces* and 2 *bottom traces* were obtained from 2 different cells. *B*: responses of a projection neuron to saturating concentration (1 mM) of GABA, 3-APS, isoguvacine, muscimol, TACA, and CACA. Timing of stimulus pulse (1 s) is depicted above current traces. In *A* and *B*, cells were voltage-clamped at -60 mV and patch pipettes were filled with high chloride Cs patch solution. *C*: dose-response relationships for GABA (\bullet), muscimol (\blacktriangledown), TACA (\blacksquare), CACA (\blacktriangle), isoguvacine (\blacklozenge), and 3-APS (\circ). Peak currents evoked by agonist at each concentration were normalized to current evoked by 1 mM agonist. Points shown are means \pm SD of 4–7 experiments. Solid lines are the fit of Eq. 1 to data (see text). Holding potential, -10 mV. Pipette, standard K-acetate patch solution.



Bicuculline methiodide did not alter the activity of GABA-gated channel ($n = 3$; data not shown). CACA evoked unitary currents when applied to outside-out patches containing a GABA-gated channel ($n = 6$, Fig. 10B). The current amplitude and slope conductance of the single channel events evoked by GABA and CACA were essentially identical—current amplitude of the open state was 1.86 ± 0.12 pA for GABA versus 1.81 ± 0.24 pA for CACA at +20 mV (SD, $n = 6$; Fig. 9C), whereas the slope conductance was 54.9 ± 0.7 pS for GABA versus 53.8 ± 0.6 pS for CACA (SE, $n = 6$; Fig. 9C, ● for GABA and ■ for CACA). This finding indicates that CACA and GABA activate the same channel, although CACA at 100 mM is less potent than 20 mM GABA.

Chloride equilibrium potential

The GABA-induced current in both cultured and acutely dissociated somata, when measured with gramicidin-perforated whole cell recording so as not to disturb E_{Cl} , reversed polarity at -73.5 ± 4.3 mV (SD, $n = 5$; Fig. 11). The estimated $[Cl^-]_i$ from the Nernst equation is 28 mM. The normal resting potential of the cells, measured with the electrode in zero current-clamp mode, immediately after establishing the whole cell recording configuration, was -62.5 ± 3.8 mV (SD, $n = 21$).

DISCUSSION

We conclude that olfactory projection neurons in the spiny lobster express an ionotropic GABA receptor that is a functional chloride channel, is antagonized by picrotoxin but not bicuculline or other competitive GABA_A receptor antagonists, and is activated by muscimol and the GABA_C receptor agonist CACA. We can further assign a functional role to this receptor with reasonable certainty. The unimposed E_{Cl} for the olfactory projection neurons of approximately -75 mV is below the experimentally determined resting potential of the cells (-62 mV). Activation of a chloride-selective GABA receptor current would therefore be expected to hyperpolarize the cell. Perfusing the intact brain with GABA increases the threshold of the projection neurons to afferent input from the olfactory (antennular) nerve, whereas PTX, but not bicuculline, has the opposite effect (Wachowiak and Ache 1997). Applying GABA directly to the somata of the projection neurons in the intact brain has no effect on the threshold of the neurons to antennular nerve stimulation (unpublished observations). It is reasonable to conclude, there-

FIG. 7. A: responses of a projection neuron to 100 μ M GABA (top), 100 μ M 3-APA (middle-top), 100 μ M imidazole-4-acetic acid (I-4AA; middle-bottom), and 100 μ M THIP (bottom). All 4 traces were obtained from same cell. B: PTX (100 μ M) reversibly blocked currents evoked by I-4AA (500 μ M, top) and 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP; 500 μ M, bottom traces) on same cell. In A and B, timing of stimulus pulse is indicated by solid bar above current traces. Holding potential, -60 mV. Pipette, KCl patch solution. C: current-voltage relationships of whole cell currents measured before (BL) and during the response induced by 1 mM GABA or 1 of the 2 following GABA analogues: I-4AA and THIP. Reversal potential of GABA- or GABA analogue-evoked current was -21.6 mV for GABA, -21.9 mV for I-4AA, and -22.1 mV for THIP, respectively. Pipette, high chloride Cs (210 mM CsCl) patch solution. Current-voltage relationships were obtained by voltage ramps (see Fig. 1).

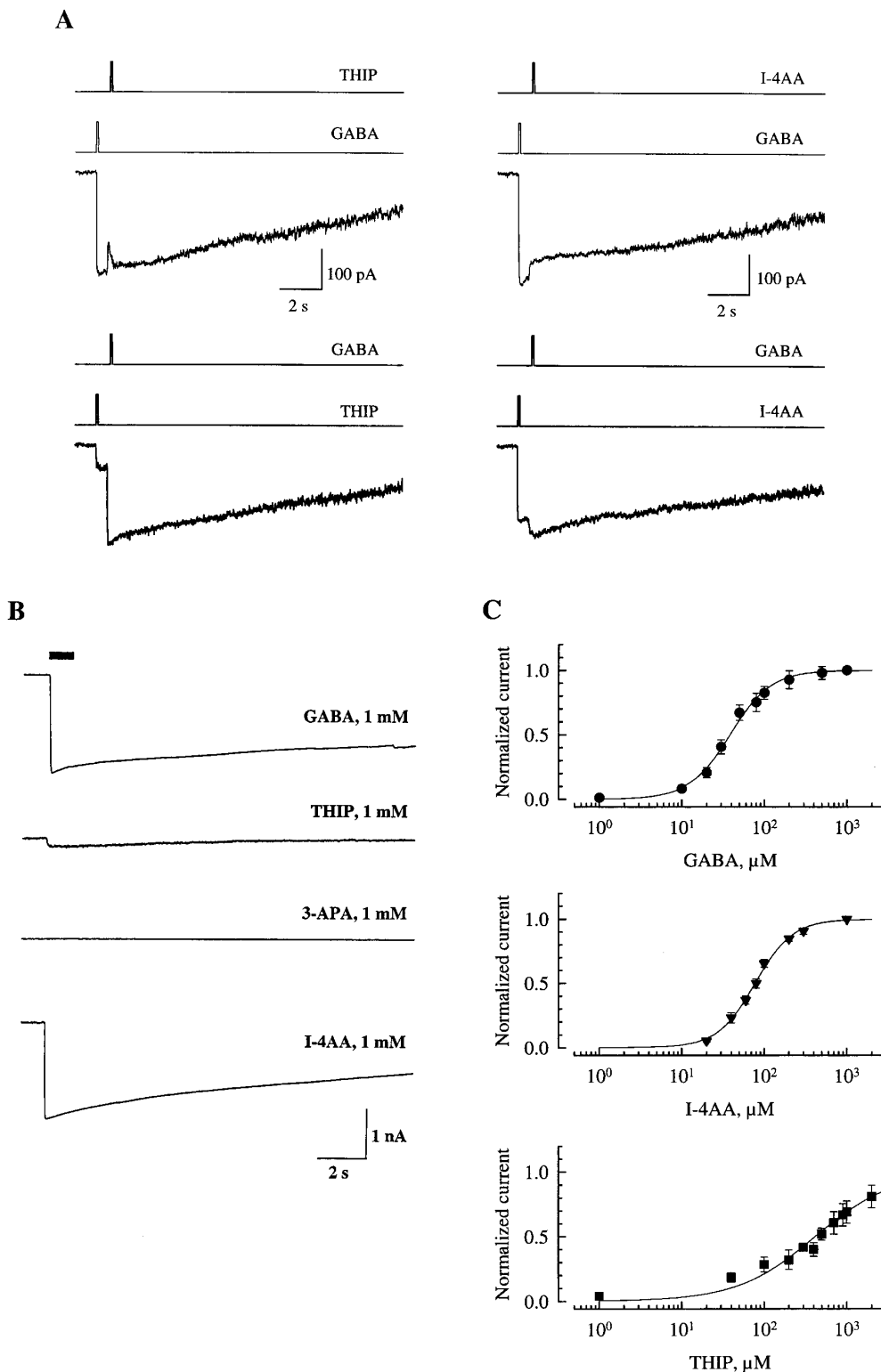


FIG. 8. *A*: responses evoked by successive application of 1 mM GABA and 1 mM THIP (or I-4AA). Two right responses and 2 left responses were induced by pairs of GABA/THIP (THIP/GABA) and GABA/I-4AA (I-4AA/GABA), respectively. Agonist type, sequence of application, and timing of stimuli are indicated above current traces. *B*: responses of a projection neuron to 1 mM GABA, THIP, 3-APA, and I-4AA. Solid bar above current traces indicates timing of stimulus. In *A* and *B*, patch pipette was filled with high chloride Cs patch solution. *C*: dose-response relationship for GABA (\bullet), I-4AA (\blacktriangledown), and THIP (\blacksquare). Except for THIP, peak currents were normalized as described in Fig. 6. For THIP, peak currents were normalized to value of maximal THIP-evoked current obtained from fitting Eq. 1 to data. Points shown are means \pm SD of 3–8 experiments. Solid lines are fit of Eq. 1 to data (see text). Holding potential, -10 mV. Pipette, standard K-acetate patch solution.

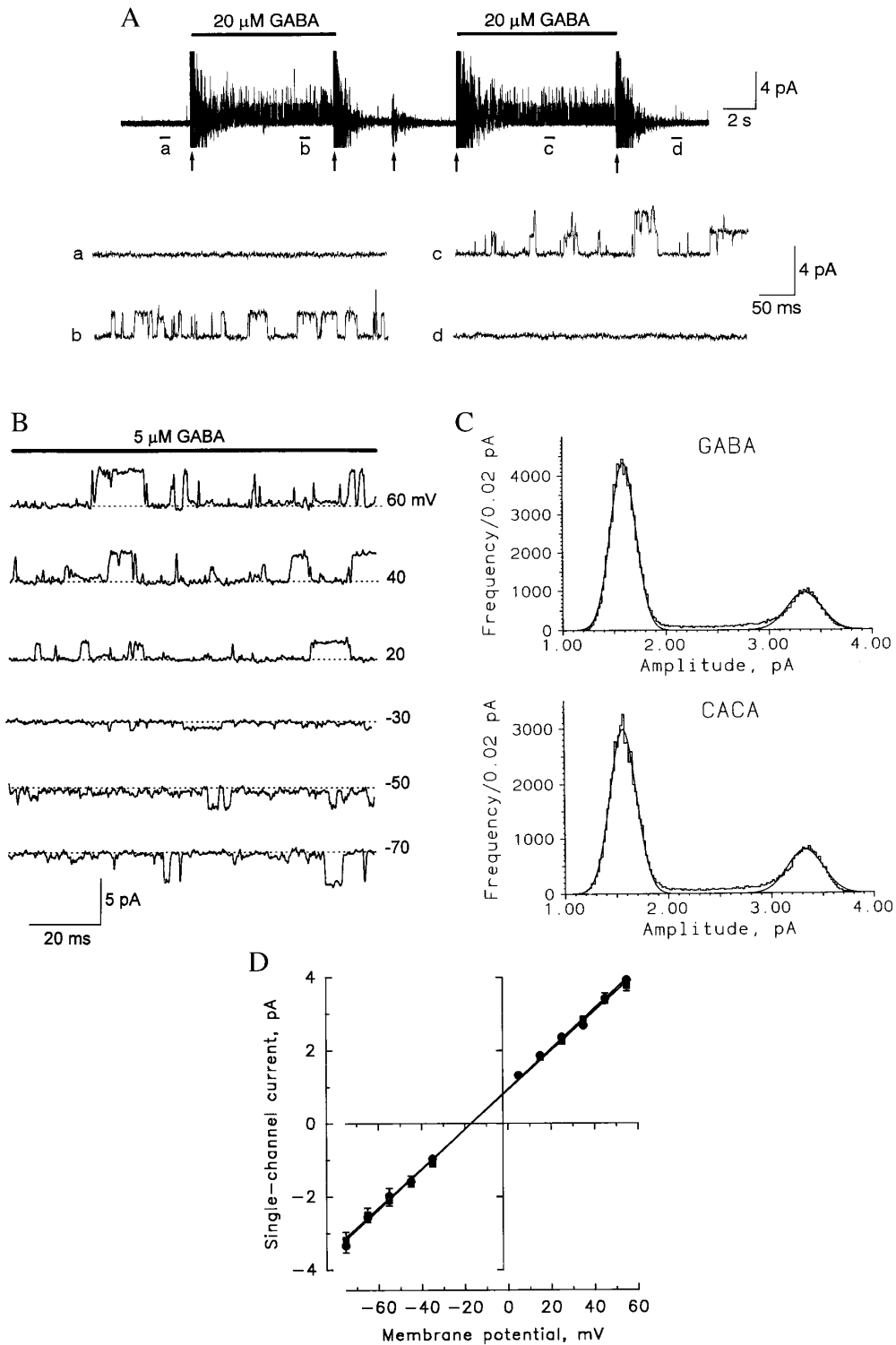


FIG. 9. GABA activates unitary currents in outside-out patches from cultured olfactory projection neurons. *A*: unitary currents activated by 20 mM GABA (bars, *top*). Note: artifacts associated with activation of perfusion system (\uparrow) are visible on this and following unitary current records. Lower traces (*a-d*) show fragments taken from *top* trace on an expanded time scale. Membrane potential, +20 mV. *B*: unitary currents from 1 patch activated by 5 mM GABA at membrane potentials indicated near each trace. *C*: amplitude histogram of unitary currents of GABA-activated (*top*; GABA, 5 μ M) and CACA-activated (*bottom*; CACA, 100 μ M) currents. Solid line is fit of sum of 2 Gaussian functions with following parameters: 1.59 pA (closed state) and 3.34 pA (open state) for GABA; 1.57 pA (closed state) and 3.33 pA (open state) for CACA. Membrane potential, +20 mV. *D*: plot of current-voltage relationship of 5 μ M GABA-activated (\bullet) and 100 μ M CACA-activated (\blacksquare) unitary currents from 6 patches plotted for open state. Bars indicate standard error. Slope conductance of open state is 54.9 ± 0.7 pS (SE) for GABA and 53.8 ± 0.6 pS (SE) for CACA between -70 and $+60$ mV. Single-channel current reverses at -18 mV, close to calculated E_{Cl} of -22.9 mV. In *A-D*, pipette solution is high chloride Cs-acetate patch solution.

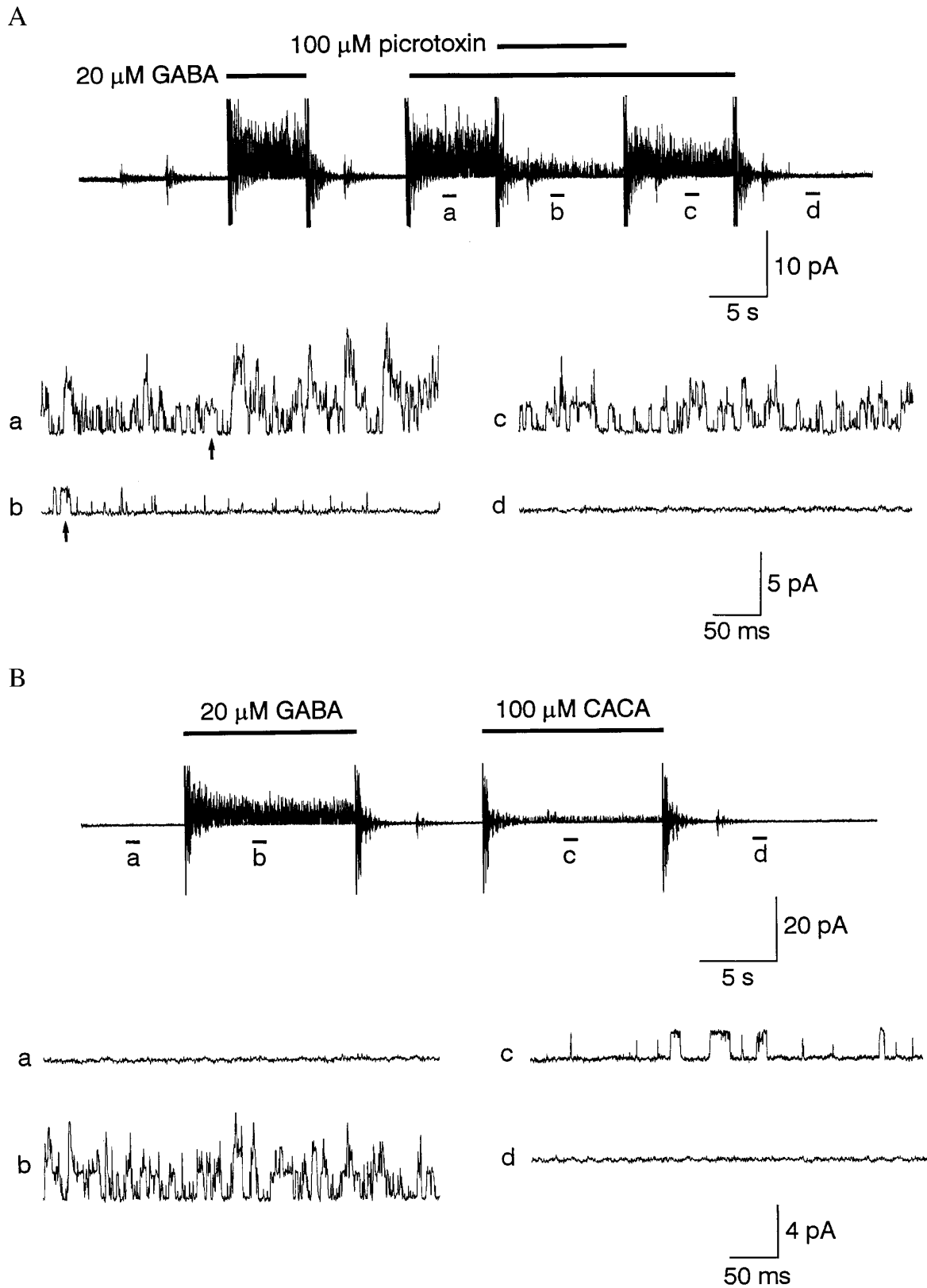


FIG. 10. *A*: picrotoxin (100 μ M, top bar) reversibly blocks GABA-activated channel in an outside-out patch from a cultured olfactory projection neuron. *Top*: unitary currents activated by 20 μ M GABA (lower bars). *Lower traces* show fragments taken from *top trace* on an expanded time scale. Note identical single channel current amplitudes in the absence and presence of picrotoxin (\uparrow). Pipette, high chloride Cs patch solution. Membrane potential, +20 mV. *B*: CACA reversibly activates GABA-activated channel in an outside-out patch from a cultured lobster olfactory projection neuron. *Top*: unitary currents activated by 20 μ M GABA (first bar) and 100 μ M CACA (second bar). *Lower traces*: fragments taken from *top trace* and shown on an expanded time scale. Membrane potential, +20 mV. Pipette, low chloride Cs-acetate patch solution.

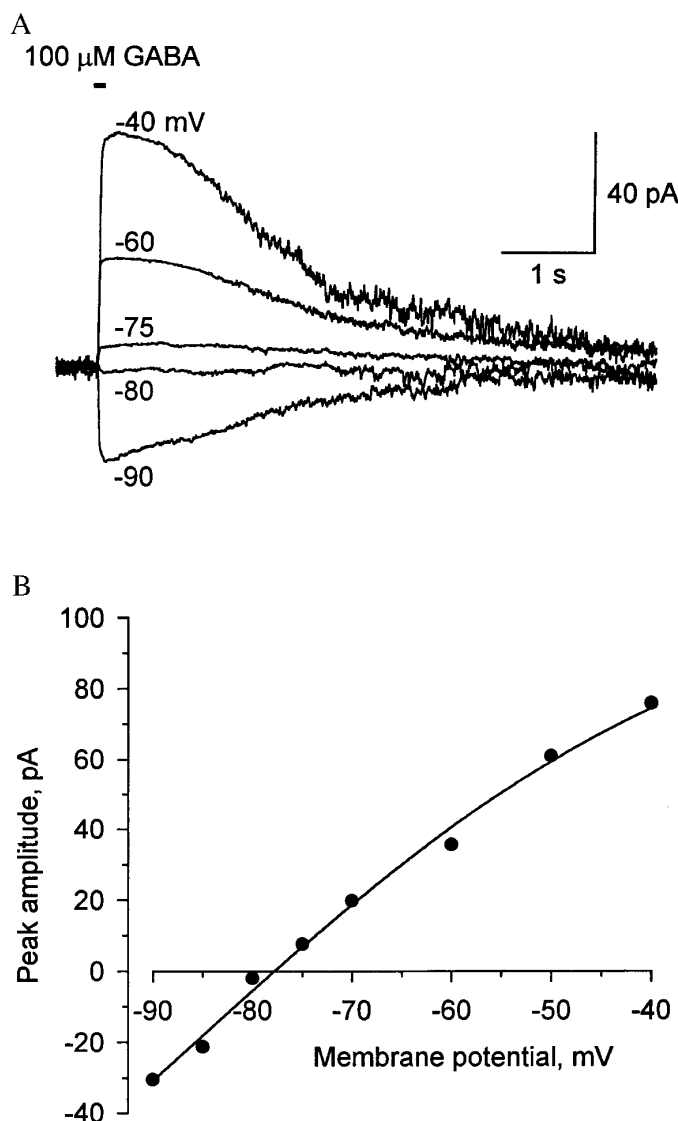


FIG. 11. Gramicidin-perforated, voltage-clamp recording from 2 acutely dissociated olfactory projection neurons. *A*: records of current evoked by 100 μM GABA (bar) from 1 neuron at membrane potentials indicated near each trace. *B*: plot of current-voltage relationship of GABA-induced current in another neuron tested under same conditions as in *A*. Pipette, KCl patch solution.

fore, that the lobster CNS receptor we describe here mediates inhibition in the olfactory neuropil of the spiny lobster.

The spiny lobster GABA receptor appears to be similar to one expressed by neurons cultured from the thoracic ganglion of the clawed lobster, *H. gammarus* (Jackel et al. 1994a,b), because the unitary and macroscopic currents and the pharmacological properties of both receptors are nearly identical. We therefore refer to this single entity as the lobster CNS receptor, without implying that this is the only type of GABA receptor in the lobster CNS. Our extended characterization of this receptor allows a more detailed comparison of the lobster CNS receptor with other known GABA receptors.

Physiologically, single-channel recordings verify that the lobster CNS receptor is a functional channel, as are other ligand-gated GABA receptors (MacDonald and Olsen 1994;

Sattelle 1992). The sensitivity of the lobster CNS channel to PTX, its insensitivity to bicuculline methiodide, and its activation by CACA indicate that the channel underlies the macroscopic GABA-induced current in the projection neurons. The Hill coefficient estimated from the dose-response function of the macroscopic current gives a lower limit of number of GABA molecules binding to the receptor close to two, which is similar to that reported for vertebrate GABA_A receptors (MacDonald and Olsen 1994) and GABA receptors on insect neurons (Sattelle et al. 1988) and crustacean muscle (Nistri and Constanti 1979). The reversal potential of the GABA-evoked current follows changes in the chloride equilibrium potential, indicating the GABA-activated channel is anion selective. The relative permeability for small anions, [SCN^- (8.51) > Br^- (5.95) > I^- (4.10) > Cl^- (1.00) > F^- (0.09)], and for large polyatomic anions, [Cl^- (1.00) > acetate (0.35) > bicarbonate (0.24) > phosphate (0.17) > propionate (0.12)], is quite similar to that of the vertebrate GABA_A-receptor channel [SCN^- (7.3) > Br^- (2.8) > I^- (1.5) > Cl^- (1.00) > F^- (0.02) and Cl^- (1.00) > bicarbonate (0.18) > acetate (0.08) > phosphate (0.023) > propionate (0.017)] (Bormann et al. 1987). The relatively high permeability of the lobster CNS receptor channel for large polyatomic anions, however, may indicate that the lobster CNS receptor channel has a larger-diameter pore than that of the vertebrate GABA_A-receptor channel. The conductance of the open state of the lobster CNS receptor channel (55 pS, present study; 56–59 pS, Jackel et al. 1994b) is close to that of the largest open state of a GABA receptor channel in crayfish muscle (68 pS) (Franke et al. 1986), but is significantly larger than the conductance of another crayfish muscle GABA receptor (35 pS) (Adelsberger et al. 1994), the vertebrate GABA_C (7 pS) (Feigenspan et al. 1993) and GABA_A channels (30 pS) (Bormann 1988) and insect GABA channels (11–25 pS) (Sattelle 1992). The relatively large conductance of the channel thus appears to be a distinct biophysical feature of the lobster CNS receptor. PTX generally is considered to be an open channel blocker that binds to a site inside of or close to the channel pore (Bormann 1988), and in the P subunits that are thought to form mammalian PTX-sensitive GABA_C receptors (Cutting et al. 1991, 1992), the proline residue at position 309 in transmembrane segment M2, which lines the pore channel, is crucial for PTX sensitivity (Enz and Bormann 1995). The essentially voltage-independent effect of picrotoxin on the lobster CNS receptor (Fig. 4B) (Jackel et al. 1994a), however, strongly suggests, although does not prove, that the binding site for picrotoxin in the lobster CNS receptor is not in the membrane's electrical field as would be predicted for GABA_C receptors. Thus, the lobster CNS receptor appears to be physiologically distinct from other ionotropic GABA receptors.

The lobster CNS receptor also appears to differ pharmacologically from vertebrate GABA_A and GABA_C receptors. The lobster CNS receptor is insensitive to the competitive GABA_A antagonists, bicuculline and (+)-hydrastine (tested here), and SR-95531 (Jackel et al. 1994a). Further, the lobster receptor is unaffected by the benzodiazepine diazepam and the barbiturate phenobarbital (Jackel et al. 1994a), both of which enhance the activation of vertebrate GABA_A receptors and at least some insect CNS GABA receptors

(Lees et al. 1987; Sattelle et al. 1988). THIP, a GABA analogue that is more potent than GABA in activating GABA_A receptor (Alger and Nicoll 1982; Krosggaard-Larsen et al. 1977) is only a partial agonist for the lobster CNS receptor. Jackel et al. (1994b) proposed that the lobster CNS receptor is similar to the vertebrate GABA_C receptor, based on its insensitivity to bicuculline and its activation by the selective vertebrate GABA_C agonist CACA (Drew et al. 1984; Feigenspan et al. 1993). Although GABA_C receptor agonists such as muscimol, TACA, and CACA (see review Bormann and Feigenspan 1995), activate the lobster CNS GABA receptor, we show that it also is activated by the vertebrate GABA_C antagonists, isoguvacine, THIP, 3-APS, and I-4AA (Kusama et al. 1993; Qian and Dowling 1994; Woodward et al. 1993). The agonist potency sequence for the lobster CNS receptor—TACA > muscimol > GABA > I-4AA > isoguvacine > 3-APS > CACA > THIP—is different from that of the vertebrate GABA_C receptor—muscimol > TACA = GABA ≫ CACA (Zhang and Slaughter 1995). Thus, we conclude that the lobster CNS receptor is pharmacologically distinct from the vertebrate GABA_C receptor, as well as from other GABA receptors heretofore characterized in vertebrates and insects.

The biophysical and pharmacological properties of the lobster CNS receptor also distinguish it from some GABA receptors previously identified in crustaceans (see Albert et al. 1986; Marder and Paupardin-Tritsch 1978; Nistri and Constanti 1979). However, we cannot ascertain whether the lobster CNS receptor is distinct from some other, partially characterized crustacean GABA receptors. PTX-sensitive, bicuculline-insensitive GABA receptors, for example, occur in the lobster cardiac ganglion (Kerrison and Freschi 1992), lobster and stomatopod stomatogastric ganglia (Cazalets et al. 1987; Tazaki and Chiba 1994), and crayfish muscle (Franke et al. 1986). Indeed, given that the lobster CNS receptor occurs in spiny lobster brain and clawed lobster thoracic ganglia, it may be expressed in a variety of crustacean excitable cells. Toward this end, a cDNA clone having highest homology to mammalian GABA_A receptors recently has been isolated from lobster brain (T. S. McClintock, personal communication). Given the apparent ubiquity of the lobster CNS receptor, it may be reasonable to expect that the cloned receptor is a subunit of the lobster CNS receptor we report here. If so, the unique properties of the lobster CNS receptor can be characterized further at the molecular level to offer insight into the structure and function of GABA receptors.

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