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$GABA_B$ RECEPTOR-MEDIATED MODULATION OF CUTANEOUS INPUT AT THE CUNEATE NUCLEUS IN ANESTHETIZED CATS

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Abstract—This study examined the modulatory influence exerted by $GABA_B$ receptors on the transmission of cutaneous afferent input to cuneate nucleus neurons in anesthetized cats. Electrical stimulation at the center of a receptive field activated cuneate nucleus cells at latencies of ≤7 ms whereas stimulation at neighboring sites (receptive field edge) increased the response latency. Extracellular recording combined with microiontophoresis demonstrated that $GABA_B$ receptors are tonically active. Blockade of $GABA_B$ receptors prolonged sensory-evoked response durations and decreased times of occurrence of successive bursts whereas the agonist baclofen suppressed both these effects. Ejection of baclofen delayed the evoked response from the receptive field edge with respect to the receptive field center response and inhibited responses from the receptive field edge more effectively than responses from the receptive field center. From these results it is concluded that activation of $GABA_B$ receptors precludes cuneate cells from reaching firing threshold when afferent inputs are weak, spatially modulate cuneate nucleus excitability, play a major role in temporal pattern of discharges, and shape cutaneous receptive fields. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: $GABA_B$-mediated inhibition, somatosensory system, cutaneous transmission, dorsal column nuclei, iontophoresis, cat.

The primary afferent fibers entering the cuneate nucleus (CN) release glutamate (Galindo et al., 1967; De Biasi et al., 1994; Popratiloff et al., 1997; Deuchars et al., 2000; Núñez and Buño, 2001) which when microiontophoresed evokes high-frequency trains of closely-spaced action potentials or bursting responses in hair- and touch-sensitive CN cells (Galindo et al., 1968). These cells also discharge bursts spontaneously or in response to primary afferent stimulation. In the latter case, the response of hair-sensitive cells is usually formed by a single burst of two to seven spikes with inter-spike intervals ranging from 1 to 4 ms. In contrast, the light touch-sensitive cells respond to skin stimulation generating a varying number of spikes per burst along the stimulating period (Soto et al., 2004). The latter components of the bursting discharges in both classes of cells are induced through relatively weaker mechanisms since they are blocked more easily by iontophoretic ejection of GABA (Galindo et al., 1968; Aguilar et al., 2002, 2003; Soto et al., 2004) and selectively suppressed by strychnine (Aguilar et al., 2002).

The underlying mechanisms of bursting have been attributed to: i) polysynaptic excitation (Amassian and De Vito, 1957), ii) a powerful synaptic action of primary afferent fibers (Andersen et al., 1964), and iii) intrinsic membrane properties (Galindo et al., 1968; Canedo et al., 1998; Reboreda et al., 2003). Furthermore, other mechanisms may also influence bursting activity at the dorsal column nuclei (DCN: cuneate and gracilis). A low-threshold mechanosensitive pathway also reaches the DCN through a spinal cord relay in the rat (Giesler et al., 1984), cat (Uddenberg, 1966; Angaut-Petit, 1975; Rustioni, 1973, 1974; Brown and Fyffe, 1981; Pierce et al., 1990), raccoon (Dick et al., 2001) and monkey (Rustioni et al., 1979; Cliffer and Willis, 1994). These fibers run in the dorsal column and can modulate the firing activity of DCN neurons (Dykes and Craig, 1998; Dick et al., 2001) including their bursting activity. Finally, presynaptic mechanisms can also modulate postsynaptic bursting by regulating the amount of neurotransmitter release.

GABA and glycine are the inhibitory neurotransmitters present in the CN (Galindo et al., 1967, 1968; Kelly and Renaud, 1973a,b; Hill et al., 1976). Inhibition shapes the cutaneous receptive field (RF) of cuneate neurons (Canedo and Aguilar, 2000; Aguilar et al., 2002, 2003; Soto et al., 2004), and the enlargement of RFs by the *in vivo* application of $GABA_B$ receptor antagonists (Schwark et al., 1999) indicates that $GABA_B$ receptors mediate some of this inhibition. The $GABA_B$ receptors are bicuculline (BiCu)-resistant (Hill and Bowery, 1981; Newberry and Simmonds, 1984) and appear to be restricted to the presynaptic primary afferent terminals in the DCN of young rats (Deuchars et al., 2000).

Since the role of $GABA_B$-mediated inhibition influencing somatosensory processing at the DCN remains poorly understood, the present work was aimed to study the influence that the $GABA_B$ receptors may have on the mechanisms of postsynaptic bursting and on shaping the RF of cuneate cells driven by cutaneous stimulation.

EXPERIMENTAL PROCEDURES

General

All procedures conformed to the Spanish Physiological Society, the International Council for Laboratory Animal Science, and the European Union Council Directive (86/609/EEC), and all efforts

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Abbreviations: BiCu, bicuculline; CL, cuneolemniscal neuron; CN, cuneate nucleus; DCN, dorsal column nuclei (cuneate and gracilis); ML, medial lemniscus; nCL, noncuneolemniscal neuron; RF, receptive field.

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were made to minimize the number of animals used and their suffering. Data were obtained from 20 anesthetized and paralyzed cats of either sex weighing 2.6–4.2 kg. Surgical anesthesia was induced with ketamine HCl (10–20 mg/kg i.m.) and continued with α-chloralose (60 mg/kg i.v., $n=12$) or sodium pentobarbital (35 mg/kg i.v., $n=8$). There was no evidence that choice of anesthetic affected the results obtained. Additional doses of anesthesia (1/2 of a full dose) were regularly administered every 5–7 h. The depth of anesthesia was evaluated by continuously monitoring the heart rate (maintained around 120 beats/min), the electrocorticogram (ECoG; digitally filtered at a frequency band-pass of 1–50–100 Hz) and by observing the state of the pupil. High-amplitude and low-frequency electrocorticographic waves were taken as sign of adequate anesthesia, and dilated pupils or pupils reacting rapidly to electrical stimuli were considered to reflect inadequate anesthesia in which case a supplementary half of a full dose of either anesthetic was immediately injected. Tracheal and venous cannulae were inserted; the animal was positioned in a stereotaxic frame and artificially ventilated. A bilateral pneumothorax was routinely performed, the expired CO_2 was maintained at $4\pm0.3\%$, a pH-balanced solution of 5% glucose in physiological saline was continuously infused (4 ml/h i.v.), and the temperature was maintained near 37.5 °C by a thermostatically controlled electric blanket and an overhead radiant heat lamp.

The foramen magnum was exposed, the posterior arch of the atlas and the occipital bone were resected to uncover the cerebellar vermis. The dura and arachnoid were then removed to insert recording electrodes in the middle CN, from the obex to 3 mm caudal to it. A bipolar stimulating electrode stereotaxically placed in the contralateral medial lemniscus (ML) at Horsley-Clarke coordinates A2, L4.5, H-5 served to antidromically identify cuneolemniscal (CL) cells according to standard criteria, including in all cases the collision test (Figs. 1B and 3A). Finally, a concentric bipolar electrode was lowered to a depth of 1–1.5 mm in the lateral tip of the cruciate sulcus to continuously monitor the electrocorticographic activity.

Extracellular recording and iontophoresis

Five-barreled pipettes attached to a mechanical microdrive were used for extracellular recording and iontophoretic ejection of drugs. Standard controls for pH and current balancing were performed. The center barrel of each five-barrel electrode was filled with 3 M NaCl for recording, one barrel was used for current balancing, and each of the remaining three barrels was filled with an aqueous solution of one of the following drugs: β-*p*-chlorophenyl-GABA (baclofen; 50 mM, pH 4; $GABA_B$ receptor agonist), the phosphonic derivative of baclofen (phaclofen; 50 mM, pH 4; $GABA_B$ receptor antagonist); *RS*-3-amino-2-(4-chlorophenyl)-2-hydroxypropyl-sulfonic acid (2-OH-saclofen; 10 mM, pH 4; $GABA_B$ receptor antagonist); 3-amino-propyl (diethoxymethyl) phosphinic acid (CGP 35348; 50 mM, pH 4; $GABA_B$ receptor antagonist), BiCu methiodide (20 mM, pH 4, $GABA_A$ receptor antagonist). Three different $GABA_B$ antagonists were used for comparison. The dose (current)/effects were similar for all them. Positive ejecting DC currents were in the range of 10–50 nA typically administered in 5–10 nA steps of increasing current while monitoring changes in sensory-evoked activity. The currents used for baclofen were established by observing increases in the latency and decreases in the number of spikes per burst on the cutaneous-evoked activation of single-neurons; those for $GABA_B$ antagonists were selected by measuring the current level necessary to reverse the effect of iontophoresed baclofen. When not in use, each drug barrel was subjected to a retaining 15–25 nA DC negative current to prevent unwanted diffusion from the pipette.

After a cell was well isolated from the background, the ML was stimulated using rectangular pulses of 0.05 ms duration and up to 1 mA intensity. The cells were classified in two subgroups according to their antidromic (CL neurons) or non-antidromic (noncune-

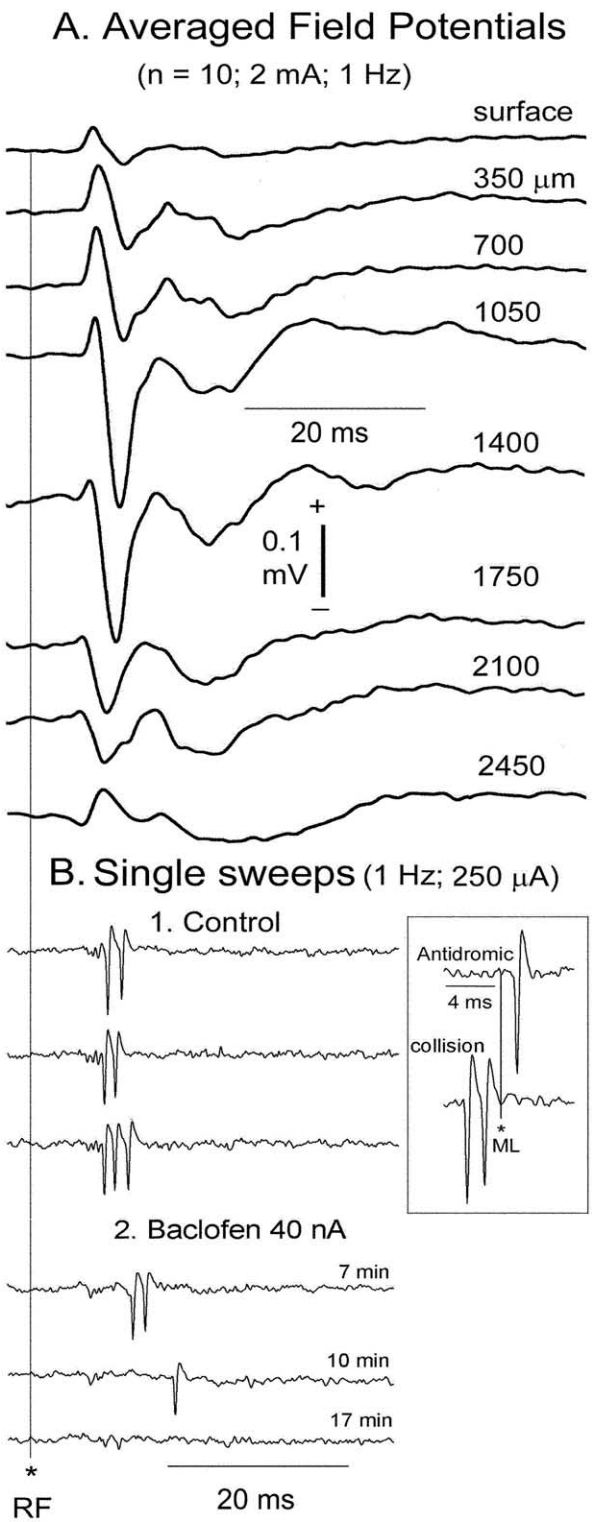

Fig. 1. Experimental procedure. The effects of $GABA_B$ agonist and antagonists were observed at a depth of 1000–1500 μm from the dorsal surface, where the negativity of field potentials to skin stimulation was maximal (A), and iontophoretic ejection of baclofen suppressed the sensory-evoked response of cuneate neurons. An example for a CL cell is shown in B (antidromic identification at the inset at a higher gain). The time of stimulation is signaled by asterisks.