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A direct nicotinic receptor-mediated inhibition recorded intracellularly *in vitro*

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ACETYLCHOLINE activates both nicotinic and muscarinic receptors in the central nervous system[1]. Although the action of acetylcholine at muscarinic receptors has been well characterized, relatively little is known at the cellular level concerning nicotinic receptor stimulation in brain. Central nicotinic receptors have been implicated in Alzheimer's disease[2], seizure activity[3], the generation of slow-wave theta rhythm in the hippocampus[4] and the potential abuse liability of nicotine[5]. At the neuronal level, nicotinic agonists have been most often associated with postsynaptically mediated excitation and membrane depolarization at various sites, including Renshaw spinal motoneurons[6], locus coeruleus[7] and the medial habenular nucleus[8]. Nicotine acting presynaptically can produce either excitation or inhibition indirectly through the release of endogeneous transmitters or modulators[9-12]. Whereas a direct inhibitory effect of nicotine has been suggested by one *in vivo* extracellular recording study in rat cerebellar Purkinje neurons[13], the mechanism(s) underlying this action is not yet known. We now report our findings obtained using *in vitro* intracellular methods in a submerged brain slice preparation in which application of nicotinic agonists to rat dorsolateral septal neurons reveal a direct membrane hyperpolarization mediated by an increase in potassium conductance.

The dorsolateral septal nucleus (DLSN) lies within the septohippocampal circuitry and is believed to play a part in integrating afferent electrical activity arising from the hippocampus before terminating in the medial septum/nucleus of Diagonal Band of Broca complex (MS/DBB)[14-16]. The MS/DBB is, in turn, involved in the generation and maintenance of hippocampal theta rhythm[17-19].

Several lines of evidence point to a role for afferent cholinergic pathways in the function of lateral septal nuclei. Acetylcholinesterase staining[20,21] as well as nicotinic and muscarinic binding sites[22-25] are evident in this region. Additionally, immunohistochemical studies indicate that the DLSN receives a cholinergic innervation from the laterodorsal tegmental[26] and pedunculopontine nuclei[27]. Consistent with these anatomical findings, electrophoretic application of acetylcholine (ACh) *in vivo* depresses spontaneous neuronal activity and electrically evoked field potentials in lateral septum, suggesting that ACh may serve to inhibit synaptic transmission in this region[28,29]. Also, recent studies from this laboratory, using *in vitro* intracellular recording methods, have provided direct evidence that neurons in the DLSN are cholinoceptive[30,31]. These latter findings indicate that cholinergic agonists produce effects which are mediated in part at pre- and postsynaptic M_1 muscarinic receptors in the DLSN. The responses to non-selective cholinergic agonists such as ACh or carbachol, however, were not completely antagonized by atropine, implying the presence of nicotinic receptors. In this study we investigated the membrane effects and ionic mechanism underlying nicotinic receptor activation in the DLSN using ACh (tested in the presence of 5 μM

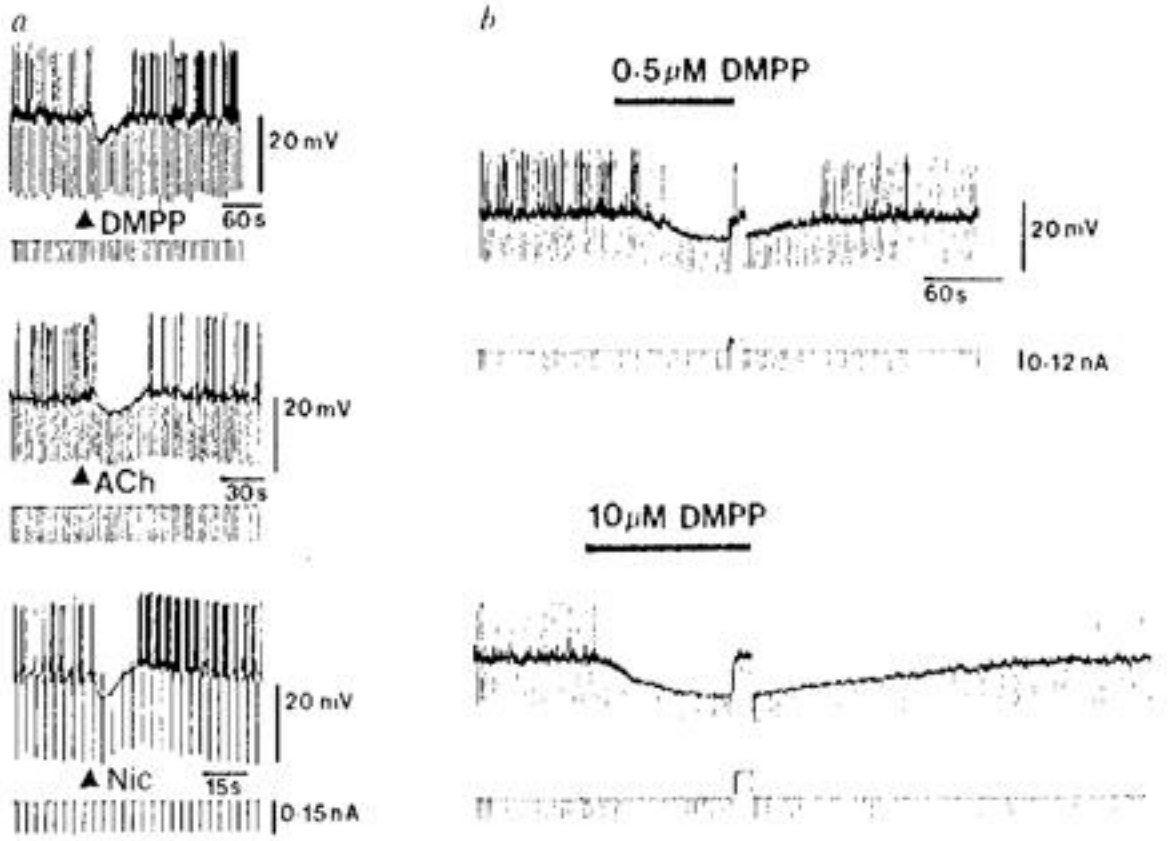

FIG. 1 Effects of DMPP, ACh and nicotine (Nic) on the membrane potential and input conductance of DLSN neurons. *a*, Pressure application of DMPP (10 mM, top); ACh (100 mM, in the presence of 5 μM atropine to block muscarinic receptors, centre); and Nic (100 mM, bottom) produced membrane hyperpolarizations (4–6 mV) lasting 20–60 s. A small amplitude membrane depolarization follows the prominent Nic-induced membrane hyperpolarization. The neurons resting membrane potential was −56 mV (top), −59 mV (centre), and −58 mV (bottom), respectively. *b*, The effect of DMPP is concentration-dependent. Bath-applied DMPP (0.5 μM) produced a 7 mV membrane hyperpolarization accompanied by decreased input resistance. Superfusion of 10 μM DMPP, in the same cell, produced a larger amplitude hyperpolarization (12 mV). The decrease in membrane resistance and inhibition of firing persisted during return of membrane potential to control level from the peak of hyperpolarization by injection of a depolarizing DC current. Upward deflections in upper trace represent action potentials, whereas downward deflections represent voltage responses to constant current injection, shown in lower trace. Resting membrane potential, −60 mV.

METHODS. Rat septal brain slices were prepared as previously described[35]. Male Sprague-Dawley rats weighing 150–250 g were decapitated, the brain rapidly dissected out and placed in cold Krebs solution of the following composition (mM): NaCl, 117; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; glucose, 11.5; $NaHCO_3$, 25; pre-bubbled with 95% O_2 and 5% CO_2. Transverse blocks of tissue containing the septum were serially cut on a Vibroslice (Campden Instrument) into 500 μm sections. A single slice was placed in the recording chamber and superfused with oxygenated Krebs solution warmed to 32 ± 1 °C. Conventional intracellular recording methods were employed using glass micropipettes (75–110 MΩ) filled with 4 M potassium acetate or 2 M potassium chloride. Hyperpolarizing current pulses (0.1–0.15 nA) were injected into the recording pipette by commands generated through an Axoclamp (Axon Instruments) amplifier. Orthodromic stimuli were delivered with square-wave pulses (5–20 V, 0.15 ms) through concentric bipolar electrodes placed focally within the DLSN. DMPP (5–10 mM), ACh chloride (100 mM) and nicotine hydrogen tartrate (100 mM) were applied by pressure-ejection (15–20 psi, 20–50 ms; Picospritzer, General Valve Corporation) through a glass micropipette (tip diameter 20–35 μm) positioned in solution within 200–300 μm from the recording electrode above the surface of the slice. By this method of application the agonist was diluted by a factor of about 1/100. All other drugs were freshly dissolved in Krebs buffer and applied by superfusion. Atropine (5 μM) was included in the bathing medium to block muscarinic receptor-mediated effects during ACh-application. Only one neuron was obtained from each brain slice for pharmacological testing.