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Neuroscience Letters 309 (2001) 77–80

Neuroscience Letters

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Effects of acetylcholine and GABA on neurons in the area postrema of *Suncus murinus* brainstem slices

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Received 18 December 2000; received in revised form 23 May 2001; accepted 4 June 2001

Abstract

Slices (400 μm) containing the area postrema were prepared from three Japanese house musk shrews. Data reported are from 16 representative units. Units fell into one of three separate groups with spontaneous firing rates of 36–41/s, 16–18/sec. and 6–8/sec., possibly reflecting different set points of an endogenous pacemaker. Most neurons did not respond to ionophoresis of GABA or ACh. In those that did, ACh briefly increased spike frequency in a dose-dependent manner. The firing pattern suggests that the membrane potential was depolarized strongly, coupled with an extremely large conductance increase. Bath perfusion of curare, but not atropine, inhibited the response. Ionophoresis of GABA caused strong inhibition. The data show Suncus AP contains neurons that are sensitive to nicotine and GABA and may be emetogenic.

Keywords: Acetylcholine; Area postrema; Atropine; Brainstem slices; Curare; GABA; Ionophoresis; *Suncus murinus*

Suncus murinus, the Japanese house musk shrew, is an animal that exhibits an emetic response with many similarities to that seen in humans [3,9,13,17,18,19]. Historically, emesis evolved as a defensive reflex to eliminate ingested toxic foods or chemicals. Chemotherapeutic agents and radiation also are emetogenic in Suncus and this species is particularly prone to motion sickness [9,13,23].

The area postrema (AP), located at the obex of the fourth ventricle, is known as the chemoreceptor trigger zone for emesis. This is due to the fact that similar to the other circumventricular organs, the AP is outside the blood–brain barrier. Therefore its cells are exposed to circulating chemicals including endogenous substances, and exogenous and metabolized toxins in the blood circulation. Additionally, it is strategically positioned to sample constituents of the cerebrospinal fluid. Ablation of the AP in all species studied, including man, renders them refractory to many heretofore emetic agents [1,2,4,5,6,7,10,12,14,16].

It has been reported that nicotine induces emesis in Suncus [13,19,23]. This is inhibited by fentanyl, an NK1 receptor antagonist [19]. However, the localization and functional characteristics of acetylcholine (ACh) receptors in this species are unknown. For these reasons, as a preliminary step in determining physiological and pharmacological mechanisms of emesis and/or the relation between motion sickness and endogenous substances, electrophysiological studies were initiated using slices of the Suncus area postrema. The first question was to establish whether acetylcholine excites AP neurons in this species, and if so to determine whether the receptors are nicotinic or muscarinic.

Under ether anesthesia, three *Suncus murinus* (36.0, 36.5, 61.2 gm) were euthanized by cervical dislocation. In each, the brain stem and cerebellum were rapidly removed to cold Krebs–Ringer solution, saturated by 95% O_2 and 5% CO_2. They then were fixed on a stage in a vibratome; a single 400 μm coronal slice was obtained containing the dorsal vagal complex including the AP. The small dimensions of the AP precluded obtaining additional slices. After 2 h pre-incubation, a slice was mounted on a fenestrated Plexiglas plate in a submerged recording chamber and perfused with oxygenated Krebs–Ringer solution at 34°C at about 3 ml/min. Single unit discharges were recorded with a glass micropipette filled with Krebs–Ringer solution (about 10 MΩ). The electrode was protruded into the area postrema until a spontaneously firing action-potential was encountered. One second 5–60 nA ionophoretic pulses of ACh or GABA (0.5 M., pH. 3.5) were delivered through an independent double-barrelled micropipette placed in proximity to the

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0304-3940/01/$ - see front matter
PII: S0304-3940(01)02049-3

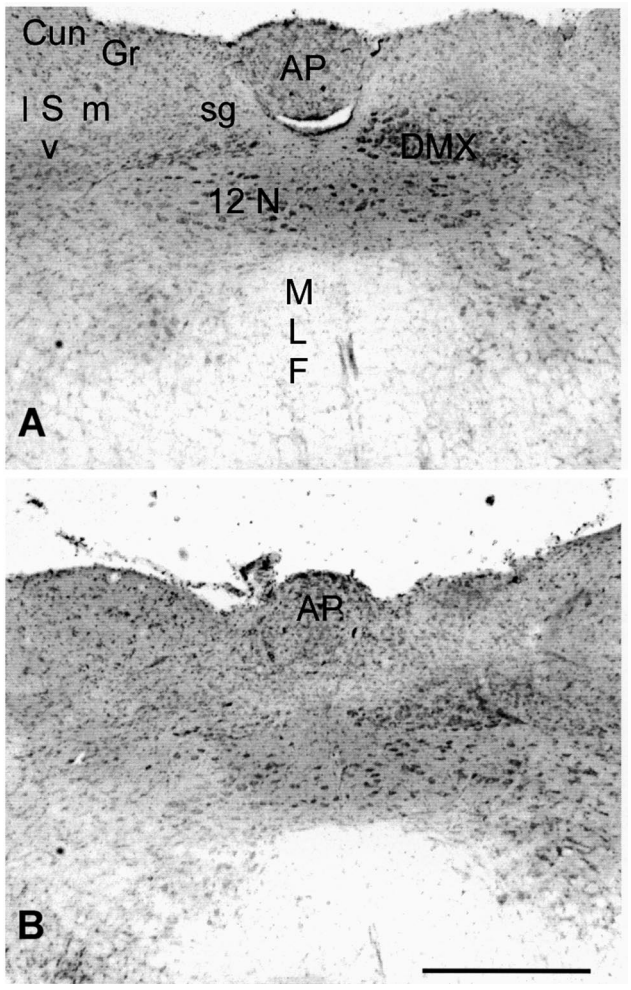

Fig. 1. Throughout its extent, the area postrema (AP) of *Suncus murinus* is positioned astride the midline. (A) At its rostral end, it sits in a depression at the obex of the 4th ventricle surrounded by other parts of the dorsal vagal complex. (B) Caudally, the AP bulges upward from the midline to form a conspicuous tubercle in the floor of the ventricle. Cun, cuneate nucleus; DMX, dorsal motor nucleus of the vagus; Gr, gracile nucleus; MLF, medial longitudinal fasciculus; S, solitary fasciculus; l, m, sg and v, lateral, medial, subgelatinosus and ventral subnuclei of the solitary complex; 12N, hypoglossal nucleus. (Thionin stain, frozen section, 20 μm thick. Calibration bar, 0.5 mm).

cell identified in this manner. Bath perfusion of 5×10^{-5} M curare or atropine was initiated after 5 min. Recordings of spontaneous activity also were made with modified Krebs–Ringer solution containing low Ca^{2+} (0.1 mM) and high Mg^{2+} (4.3 mM). Data were stored on tape.

At its rostral end the AP, roughly spherical in shape, occupies the middle of a V-shaped paramedial cleft bounded bilaterally by the solitary nuclei (Fig. 1). Caudally, the dorsal surface of the AP bulges conspicuously into the fourth ventricle. The AP is smaller than in rat and shaped somewhat differently. The configuration of the nucleus ambiguus also is slightly distinctive [22].

Single unit discharges were much more readily obtained from Suncus AP than in either rat or ferret for reasons that are unclear, but perhaps related to some topographic features. Spontaneous firings of upwards of 30 neurons were isolated in a single slice, and as many as 20 were held for a protracted period. These observations suggest that despite its smaller size, the total number of neurons in Suncus AP is not measurably different from the cell complement in the larger rat and still larger ferret.

The majority of units displayed bursts of discharges at regularly spaced intervals (Fig. 2Aa–d). However, a small number fired repetitively at a constant rate (Fig. 2 Ae). The latter exhibited what we regard as a pacemaker pattern like that seen in many species; firing rates were unchanged when modified Krebs–Ringer solution containing low Ca^{2+} (0.1 mM) and high Mg^{2+} (4.3 mM) was used to block synaptic transmission (Fig. 2B). They resembled neurons of the suprachiasmatic nucleus of the hypothalamus, the medial vestibular nucleus and neurons in other locations [8,11,21]. It should be noted that a similar firing pattern has been observed in the area postrema of rats and ferrets [15,20].

Of the approximately 60 units held for an extended period, 16 were completely analyzed. Units displayed firing rates ranging from 6 to 41 spikes/sec. Surprisingly, units fell into one of three separate groups (Fig. 2C). Neurons in group 1 had firing rates of 36–41/s ($n = 5$); group 2, 16–18/s ($n = 3$) and group 3, 6–8 spikes/sec ($n = 8$). We offer no explanation for the markedly different spike rates, but

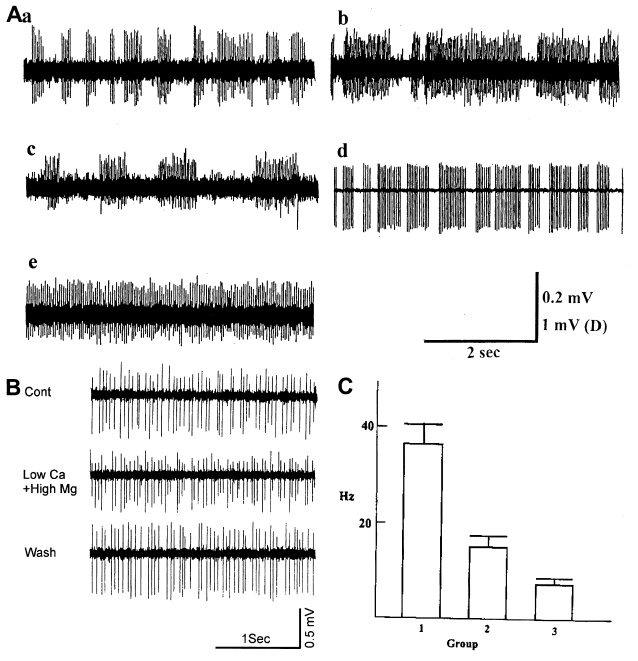

Fig. 2. Area postrema spike recordings. (Aa–d) Bursts of discharges from different neurons at regularly spaced intervals. (Ae) Repetitive constant rate discharges. (B) Spontaneous activity 5 min after application of Krebs–Ringer solution with low Ca^{2+} (0.1 mM) and high Mg^{2+} (4.3 mM). (C) Frequency histogram of spontaneous spikes. Group 1, $n = 5$; group 2, $n = 3$; group 3, $n = 8$. Bars denote STD.

suggest the possibility that it may reflect different set points of endogenous pacemaker activity, or possibly differences in the number of synapses upon neurons in each group. This in turn raises the possibility that each group may be engaged in detecting different changes in their immediate environment.

Most neurons (more than 80%) did not respond to ionophoretically applied ACh or GABA, but some exhibited brief excitation to ACh (Fig. 3A) and inhibition to the latter (Fig. 4). Constant rate neurons were relatively more sensitive to ACh and less sensitive to GABA than burst units, and conversely the latter were relatively more sensitive to GABA and less sensitive to ACh. We are not persuaded that these results suggest a paucity of cholinergic or GABAergic neurons in the Suncus AP. The lack of response may reflect difficulty in positioning the ionophoretic electrode sufficiently close to the small neuron being recorded, or that the particularly rich vascular supply in this structure interferes with the ionophoresis by dispersing the effluent.

Fig. 3A shows the typical dose-dependent excitation elicited by ionophoretic application of ACh. Ionophoresis at 15 nA current did not alter spike amplitude or frequency. Higher current levels, from 35 to 76 nA, produced progressively lower spike amplitudes. The firing pattern suggests that the membrane potential was depolarized strongly by ACh coupled with an extremely large conductance increase. Rudd et al. [19] have reported that nicotine-induced emesis was blocked by the infusion of tachykinin, an NK1 antagonist, into the dorsal vagal complex. To determine whether the responses were mediated by nicotinic or muscarinic receptors, the nicotinic receptor antagonist curare (Fig. 3B), or the muscarinic receptor antagonist atropine (Fig. 3C) was bath perfused at 5×10^{-5} M concentrations 5 min after recordings were started. The spontaneous firing rate in three neurons was reduced by curare, but unaffected by atropine, indicating that AP neurons must predominantly have ACh nicotinic receptors.

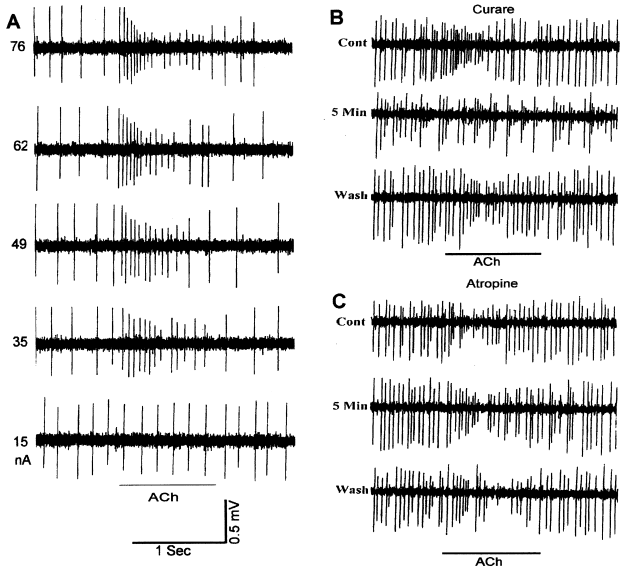

Fig. 3. Action potentials in constant rate AP neurons elicited by ACh ionophoresis (1 s, 0.5 mV). (A) Effects of altering current intensity from 15 to 76 nA. (B) Five minutes after bath perfusion with curare (5×10^{-5} M). (C) Five minutes after bath perfusion with atropine (5×10^{-5} M).

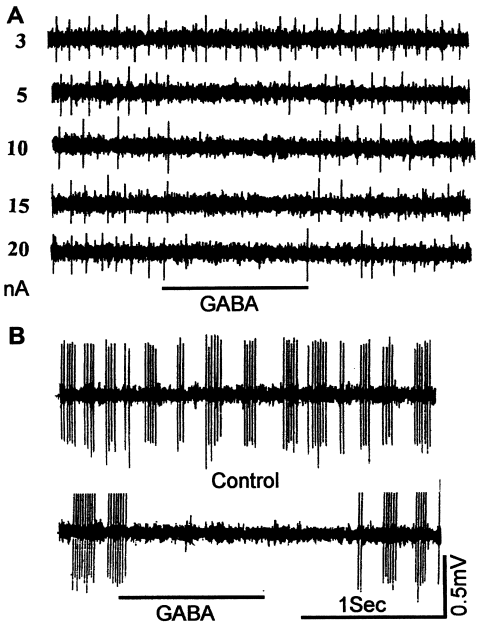

Fig. 4. Inhibitory effects of 1 s ionophoretic pulses of GABA (0.5 M., pH. 3.5). (A) Effects of increasing current intensity (3–20 nA) on regularly firing units. (B) Effect of 5 nA pulses upon burst units.

Fig. 4 shows the effects of 1 s ionophoretic pulses of GABA on spontaneous units that fired in a constant repetitive fashion as well as those that fired in regularly spaced bursts. Pulses of 3 nA did not alter the firing pattern of the former, but 5 nA caused maximal inhibition. (Fig. 4A). Burst units also were transiently inhibited by 5 nA GABA pulses (Fig. 4B).

Results of this study indicate that *Suncus murinus* contains a population of neurons in the AP that have receptors for ACh and GABA and may be related to emesis specifically induced by injection of nicotine [13,19,23]. At least some of the neurons are pacemakers.

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