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INACTIVATION OF PREFRONTAL CORTEX ABOLISHES CORTICAL ACETYLCHOLINE RELEASE EVOKED BY SENSORY OR SENSORY PATHWAY STIMULATION IN THE RAT

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Abstract—Sensory stimulation and electrical stimulation of sensory pathways evoke an increase in acetylcholine release from the corresponding cortical areas. The pathways by which such sensory information reaches the cholinergic neurons of the basal forebrain that are responsible for this release are unclear, but have been hypothesized to pass through the prefrontal cortex (PFC). This hypothesis was tested in urethane-anesthetized rats using microdialysis to collect acetylcholine from somatosensory, visual, or auditory cortex, before and after the PFC was inactivated by local microdialysis delivery of the GABA-A receptor agonist muscimol (0.2% for 10 min at 2 μl/min). Before PFC inactivation, peripheral sensory stimulation and ventral posterolateral thalamic stimulation evoked 60 and 105% increases, respectively, in acetylcholine release from somatosensory cortex. Stimulation of the lateral geniculate nucleus evoked a 57% increase in acetylcholine release from visual cortex and stimulation of the medial geniculate nucleus evoked a 72% increase from auditory cortex. Muscimol delivery to the PFC completely abolished each of these evoked increases (overall mean change from baseline=−7%). In addition, the spontaneous level of acetylcholine release in somatosensory, visual, and auditory cortices was reduced by 15–59% following PFC inactivation, suggesting that PFC activity has a tonic facilitatory influence on the basal forebrain cholinergic neurons. These experiments demonstrate that the PFC is necessary for sensory pathway evoked cortical ACh release and strongly support the proposed sensory cortex-to-PFC-to-basal forebrain circuit for each of these modalities.

Key words: microdialysis, muscimol, cholinergic basal forebrain, neocortex, systems neuroscience, cortical circuitry.

Acetylcholine (ACh) is a neurotransmitter that has been implicated in the regulation of a variety of higher cortical functions, including plasticity, working memory and attention (Rasmusson, 2000, 2006; Sarter et al., 2003; Dalley et al., 2004). Almost all cortical ACh is extrinsic and released by the terminals of cholinergic neurons whose cell bodies are located in the basal forebrain (BF). The extracellular concentration of ACh within the cortex is commonly interpreted as indicating the level of activity of these cholinergic neurons. Studies measuring cortical ACh have demonstrated that sensory stimulation evokes an increase in release from sensory cortical areas and does so with a degree of regional specificity; for example, visual stimulation causes much greater ACh release in visual cortex than in nonvisual areas (Collier and Mitchell, 1966; Fournier et al., 2004; Laplante et al., 2005).

The mechanisms and pathways by which sensory stimulation causes this modality-specific increase in ACh release are unknown. Presynaptic facilitation of the cortical cholinergic terminals by the thalamocortical afferents appears unlikely (Materi and Semba, 2001). A more likely alternative is that the increased release reflects increased firing of the cholinergic BF neurons. The pathway by which sensory input reaches the cholinergic BF neurons is unclear; traditional anatomical tracing methods have not revealed any extensive projections from sensory relay nuclei to the BF (Grove, 1988; Semba et al., 1988; Záborszky et al., 1991). A recent proposal is that sensory stimulation first activates the sensory cortex, which then activates the prefrontal cortex (PFC), which in turn activates the cholinergic BF neurons (Záborszky et al., 1997), an example of "top-down" processing (Sarter et al., 2001). PFC was proposed as an important component of this circuit because it is one of the few cortical areas that projects to the BF (Gaykema et al., 1991; Záborszky et al., 1997; Vertes, 2004) and it receives corticocortical projections from primary and association sensory cortices (van Eden et al., 1992; Condé et al., 1995). This hypothesis was supported by the demonstration that neurons in distinct regions of PFC are activated by visual or somatosensory cortex stimulation, and that stimulation of these PFC regions elicits firing of BF neurons (Golmayo et al., 2003). In addition, chemical stimulation of PFC produces an increase in ACh release in the parietal cortex (Nelson et al., 2005), consistent with a functional connection from PFC onto cholinergic BF neurons.

A specific prediction of this proposed circuit is that interrupting the pathway by inactivating the PFC should abolish sensory-evoked ACh release in sensory cortices. We tested this prediction by measuring the evoked release of ACh from somatosensory, visual and auditory cortices before and after blocking neuronal activity in PFC by the local administration of a selective GABA-A receptor agonist, muscimol. ACh release from somatosensory cortex was evoked by peripheral stimulation in one group of animals. In three additional groups the specific thalamic nu-

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Abbreviations: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BF, basal forebrain; DLG, dorsal lateral geniculate nucleus; HPLC, high-performance liquid chromatography; MGV, ventral medial geniculate nucleus; PFC, prefrontal cortex; VPL, ventral posterolateral thalamic nucleus.

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clei for these modalities were electrically stimulated using the same stimulus parameters, to allow for more direct comparisons between the somatosensory, visual and auditory modalities. The comparison between sensory and thalamic stimulation within the somatosensory modality provided validation for this approach. The comparison of evoked ACh release before and after muscimol allowed each animal to serve as its own control, thereby removing possible confounding variables such as the effect of anesthesia and probe efficiency, which could vary between animals, or the composition of the perfusate, which was the same in both stimulation periods. Any differences in evoked ACh release between the two stimulation periods can therefore be confidently attributed to muscimol inactivation of the PFC.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (200–375 g; Charles River, St. Constant, Quebec, Canada) were used. All experimental procedures were approved by the University Animal Care Committee and were carried out in accord with the Canadian Council on Animal Care and National Institutes of Health guidelines on the ethical use of animals in research. All efforts were made to minimize the number of animals used and their suffering.

ACh release from sensory cortical regions was studied in 44 rats anesthetized with urethane (1.6 g/kg, i.p.; Sigma, St. Louis, MO, USA) and placed in a stereotaxic frame. Holes were drilled in the skull for implantation of two microdialysis probes (CMA/12; CMA Microdialysis AB, Solna, Sweden; 20 kDa cutoff; 0.5 mm o.d.): one for muscimol delivery into region M2 (secondary motor area) of PFC (anterior 3, lateral 2, ventral 2; mm with respect to bregma, Fig. 1A), and a second for ACh collection into primary somatosensory cortex (posterior 1.5, lateral 2.5, Fig. 1B), primary visual cortex (posterior 7.5, lateral 4.6, Fig. 1C), or primary auditory cortex (30° angle at posterior 4.0, lateral 6.5, Fig. 1D). All microdialysis probes had 2 mm of exposed membrane and were inserted so the entire membrane was within the cortex.

Perfusion with artificial cerebrospinal fluid (aCSF: 125 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 23 mM $NaHCO_3$, and 1.5 mM H_2PO_4) was begun immediately after inserting the microdialysis probes at a rate of 2 μl/min. In order to obtain sufficient basal amounts of ACh that were consistently measurable in the anesthetized preparation, the perfusate in the ACh collection probe also contained neostigmine methyl sulfate and atropine sulfate (Sigma) (10 μM for both drugs, except in one experiment in which 1 μM neostigmine and 0.2 μM atropine were used). The comparison of release before vs. after PFC inactivation in the same animals removed the possibility that high resting levels of ACh were responsible for any changes in ACh release resulting from muscimol treatment.

The probes were perfused for 60 min to allow equilibration, before collecting 15 15-min samples. These samples were analyzed for ACh content using high-performance liquid chromatography (HPLC; Waters, Mississauga, ON, Canada) with electrochemical detection. The procedures and solutions for HPLC were as described previously (Materi et al., 2000), except that in the present experiment the working electrode was a horseradish peroxidase–coated carbon electrode (BAS, Indianapolis, IN, USA) with an oxidation potential of +500 mV.

The probe in PFC was perfused with aCSF at a rate of 2 μl/min. At the beginning of collection sample 7, the solution was changed using a liquid switch (CMA) to aCSF containing 0.2% muscimol for 10 min and then switched back to aCSF alone. The dead space in the tubing was not a concern, as the time involved (1–1.5 min) was a small fraction of the time to the second stimulation test (60–75 min). This concentration was chosen based on findings showing a very long-lasting (several hours) inhibition of neural activity over a distance of at least 2–2.5 mm (Partsalis et al., 1995; Arikan et al., 2002; Edeline et al., 2002).

Evoked release of ACh from primary somatosensory cortex was measured in response to either peripheral stimulation of the contralateral forepaw or electrical stimulation of the ipsilateral specific thalamic nucleus (ventral posterolateral nucleus, VPL) that projects to the cortical collection site. In one group (n=7), peripheral somatosensory stimulation was delivered throughout samples 4 (before muscimol, S1) and 13 (after muscimol, S2) via two s.c. wires inserted into the contralateral forepaw at the beginning of the experiment. Constant current pulses (2 mA, 0.1 ms duration) were delivered every 30 s by a constant-current stimulator and isolation unit (Master-8, A.M.P.I., Jerusalem, Israel). ACh release from somatosensory cortex was monitored in nine rats with the stimulating electrode implanted into ipsilateral VPL (posterior 3.4, lateral 3.4, ventral 6.4, Fig. 1E). The VPL/somatosensory cortex procedure was repeated in another group of eight animals using reduced concentrations of neostigmine (1 μM) and atropine (0.2 μM). A control group (n=4) received VPL stimulation without muscimol administration. ACh release from visual cortex was measured in seven rats using thalamic stimulation of the dorsal lateral geniculate nucleus (DLG) with the electrode placed at posterior 4.7, lateral 3.8, ventral 5.0 (Fig. 1F). ACh release from auditory cortex was measured in nine rats with the stimulation electrode in the ventral medial geniculate nucleus (MGV) at posterior 5.2, lateral 3.6, ventral 6.4 (Fig. 1G). Thalamic stimulation in all cases consisted of single 0.5 mA pulses (0.5 ms duration) delivered via a concentric bipolar electrode (FHC, Bowdoin, ME, USA) every 15 s throughout samples 4 (pre-muscimol, S1) and 12 (post-muscimol, S2).

For each animal the three initial baseline samples (B1) were averaged and used to normalize the release across all samples. This removed inter-animal variability due to possible differences in probe efficiency or anesthetic level. The mean of the two samples prior to S2 provided a baseline measure of spontaneous release after muscimol (B2) and was used to calculate evoked ACh release during the subsequent stimulation sample (S2/B2).

At the end of each experiment the ACh collection and muscimol delivery probes were removed from the brain and placed in a standard ACh solution; the subsequent sample was measured with HPLC to determine probe efficiency. The animal was perfused transcardially with 0.9% buffered saline followed by 10% formalin for histological confirmation of the sites of probes and stimulation electrodes. Data from animals in which the entire probes were not within 500 μm of the intended sites were excluded from analysis.

Due to the dramatic change in evoked release observed after muscimol administration, it was necessary to ensure that muscimol did not diffuse from the PFC to the sites at which ACh was collected. Therefore, electrophysiological experiments were carried out in three additional rats. Evoked responses were recorded from somatosensory cortex, the sensory cortical area closest to PFC, before and after muscimol was delivered to the same PFC site used in the collection experiments. Field potentials were recorded using tungsten microelectrodes (FHC), amplified (AM Systems, Carlsborg, WA, USA), filtered between 1 and 500 Hz, and collected on a microcomputer at a sampling frequency of 10 kHz using a DataWave interface and software (Englewood, CO, USA). The contralateral paw was stimulated electrically every 2 s (4 mA, 0.1 ms pulses; Master-8) and evoked potentials were saved for 30 min before and 155–225 min (average 189 min) after 10 min administration of 0.2% muscimol. The amplitude of the initial negative wave of the average of 10 potentials was measured and compared before and after muscimol inactivation of the PFC.