Studies on the Diversity of Muscarinic Receptors in the Autoregulation of Acetylcholine Release in the Rodent Cerebrum Using Furan Analogs of Muscarine[a]

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Previous studies have indicated that two feedback mechanisms, one positive and the other negative, regulate the rate of acetylcholine (ACh) release.[1–3] The positive feedback system has at least three components: a muscarinic receptor (Ms), release of substance P (SP), and influx of extracellular Ca^{2+}. If the amount of ACh released from the presynaptic nerve terminal is low, the released ACh activates Ms, which stimulates the release of SP. Substance P increases Ca^{2+} influx, resulting in the release of further quantities of ACh for effective cholinergic transmission. Similarly, three components are present in the negative feedback system: a presynaptic muscarinic receptor (Mi), release of methionine enkephalin (MEK), and inhibition of Ca^{2+} influx. If the ACh in the synaptic gap is high, it activates Mi, resulting in the release of MEK. Methionine enkephalin decreases Ca^{2+} influx which decreases the rate of release of ACh from the cholinergic nerve terminal. The critical first step in both of these feedback systems is activation of presynaptic muscarinic receptors, Ms and Mi. No selective agonists have been described for presynaptic muscarinic receptors. Therefore, we studied the effects of 5-methylfurfuryltrimethylammonium (5-MFT) and 5-hydroxyfurfurltrimethylammonium (5-HMFT) on the simultaneous release of ACh, SP, and MEK from the superfused mouse cerebral slices in our search for selective agonists for Ms and Mi receptors. 5-MFT and 5-HMFT were selected for this purpose because they have diverse effects in the peripheral nervous system.[4,5]

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METHODS AND RESULTS

Mouse cerebral slices were incubated in a modified Kreb's ringer buffer containing methyl-[^{3}H]choline (0.1 mM; 0.25 μCi/mL) for 60 min. They were filtered, washed, and transferred to a microbath set up for superfusion with the above buffer containing hemicholinium-3 (10 μM). The release of [^{3}H]ACh into the superfusate was measured as a function of time.[1-3] The effects of 5-MFT and 5-HMFT on the simultaneous release of labeled [^{3}H]ACh, SP, and MEK from the superfused mouse cerebral slices were determined. The [^{3}H]ACh was measured by a radiometric method. SP and MEK were measured by selective radioimmunoassays.[6] Both spontaneous and elcctrically evoked release of [^{3}H]ACh, SP, and MEK were

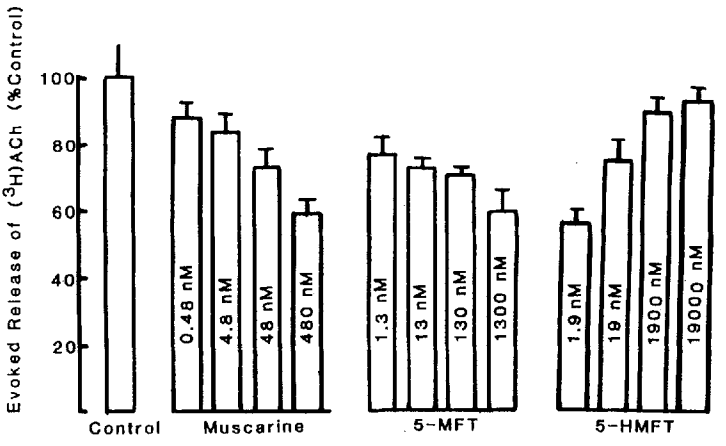

FIGURE 1. Effects of muscarine, 5-MFT, and 5-HMFT on [^{3}H]ACh-evoked release as a function of concentration. Detailed conditions for the preparation of slices, their incubation with [^{3}H]choline to form [^{3}H]ACh, and the superfusion of the slices and collection of superfusion samples for 1 h were described elsewhere.[1-3] During 30–60 min, the slices were subjected to field electrical stimulation to measure evoked release of [^{3}H]ACh in the presence and absence of 5-MFT or 5-HMFT. All values expressed as percentage of control values. Each bar is a mean ± SE from six values. Similar results are obtained for spontaneous release during 0–30 min of superfusion. The effect of muscarine and 5-MFT increased with increasing concentration, whereas the effect of 5-HMFT decreased with increasing concentration.

measured. 5-MFT (13–1300 nM) decreased spontaneous ACh release (60% of control) in a concentration-dependent manner (FIG. 1). 5-HMFT (1.9 nM) decreased the spontaneous release of ACh to 40% of control. This effect is inversely related to increasing concentrations of 5-HMFT. At 190 μM of 5-HMFT, the effect was only 15% of control. The effect of 5-MFT was antagonized by atropine (1 μM) but not naloxone (55 nM). The effect of 5-HMFT was antagonized by scopolamine (10 nM) and naloxone. 5-MFT was considerably more potent than 5-HMFT in stimulating muscarinic receptors (M) in smooth muscle. Both 5-MFT and 5-HMFT inhibited MEK release whereas 5-HMFT was more potent in causing SP release (FIG. 2). These observations indicate that autoregulation of ACh release operates through two subtypes of M, one stimulatory (Ms) and the other inhibitory (Mi). The

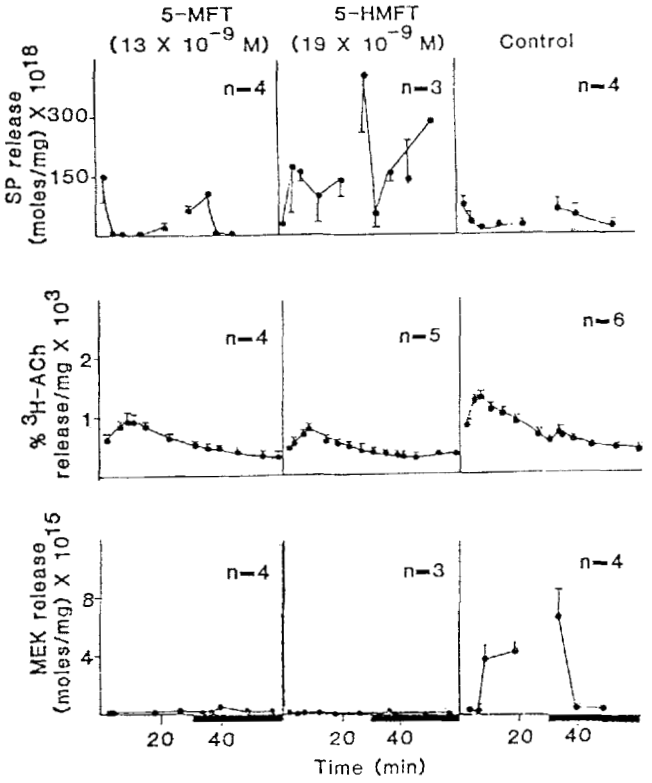

FIGURE 2. Patterns of the effect of 5-MFT (13×10^{-9} M) and 5-HMFT (19×10^{-9} M) on the simultaneous release of substance P (SP), acetylcholine (ACh), and methionine enkephalin (MEK) from mouse cerebral slices. Each point represents a mean; the vertical lines represent the standard error. Inhibition of the release of MEK predominates both with 5-MFT and 5-HMFT. Depressions in ACh release seem to trigger the enhanced SP release and depressed MEK release. Thick horizontal bars (*bottom panel*) indicate periods of field electrical stimulation.

identity of Ms and Mi receptors with M1, M2, or M3 muscarine receptor subtypes is not yet determined.[7,8]

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