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Role of the M_1 receptor in regulating circadian rhythms

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Abstract

Cholinergic stimuli are potent regulators of the circadian clock in the hypothalamic suprachiasmatic nucleus (SCN). Using a brain slice model, we have found that the SCN clock is subject to muscarinic regulation, a sensitivity expressed only during the night of the clock's 24-h cycle. Pharmacological and signal transduction characteristics are compatible with a response mediated by an M_1–like receptor. Molecular manipulation of muscarinic receptors will provide important insights as to the receptor subtype(s) regulating circadian rhythms.

Keywords: Acetylcholine; cGMP/PKG signaling; Circadian rhythms; Muscarinic receptors; Suprachiasmatic nucleus

Fundamental behaviors, such as locomotory activity *vs.* sleep, exhibit alternating patterns of expression with characteristic relationships to the day-night cycle. Patterning is controlled by the master circadian clock that resides within the suprachiasmatic nucleus (SCN) at the base of the hypothalamus. The SCN circadian clock is an endogenous, dynamic set of cellular state progressions that generates a ∼24-h timebase. Efferent signals, in turn, organize physiological, hormonal and behavioral functions into near 24-h cycles, termed *circadian rhythms*. In addition to this timekeeping role, the SCN integrates afferent signals relaying changes in external and internal temporal state. The clock restricts the timing of its sensitivity to these signals so that they communicate temporal desynchronization, readjusting the timekeeping mechanism. This gatekeeping property and the capacity for clock resetting are fundamental to appropriately orchestrating organismic functions over the day-night cycle [1].

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Acetylcholine (ACh) has long been implicated in nocturnal adjustment of circadian rhythms [2,3], although the behavioral context in which it functions is not yet established. The SCN is not intrinsically cholinergic [4], but receives direct cholinergic projections from the basal forebrain and brain stem tegmentum [5]. These brain regions regulate changes of sleep and arousal states, which occur in prominent day-night patterns that are timed by the SCN. Their cholinergic innervations of the SCN are potential feedback circuits [6].

Clock properties are preserved for at least three days in a hypothalamic brain slice containing the SCN. The neuronal ensemble within the SCN generates stable 24-h rhythms of spontaneous firing rate that peak near midday *in vitro* [7], as they do *in vivo* [8]; this peak provides an unambiguous marker of clock phase. Additionally, the SCN clock *in vitro* continues to gate its sensitivity to resetting stimuli [9,10]. Of note is the finding that clock sensitivity in the brain slice to glutamate, the neurochemical messenger transmitting environmental light signals from the eye to the SCN, matches in detail the timing and pattern of sensitivity of behavioral rhythms in animals to phase resetting by nocturnal light [11,12]. Thus, the suprachiasmatic brain slice offers an experimentally accessible preparation for investigating mechanisms that mediate cholinergic regulation of the SCN clock.

We have evaluated the direct action of the cholinergic agonist, carbachol, on the rhythm of SCN neuronal activity using extracellular electrophysiological recording techniques. We found that the SCN clock is sensitive to cholinergic stimulation such that the phase of the clock, as measured by timing of the peak in the spontaneous activity rhythm, is advanced (Fig. 1a, b). Phase advance means that clock processes jump ahead, as the timekeeping mechanism is immediately reset to a new state from which time is reckoned from that point on. Although the SCN brain slice is maintained under constant conditions *in vitro*, cholin-

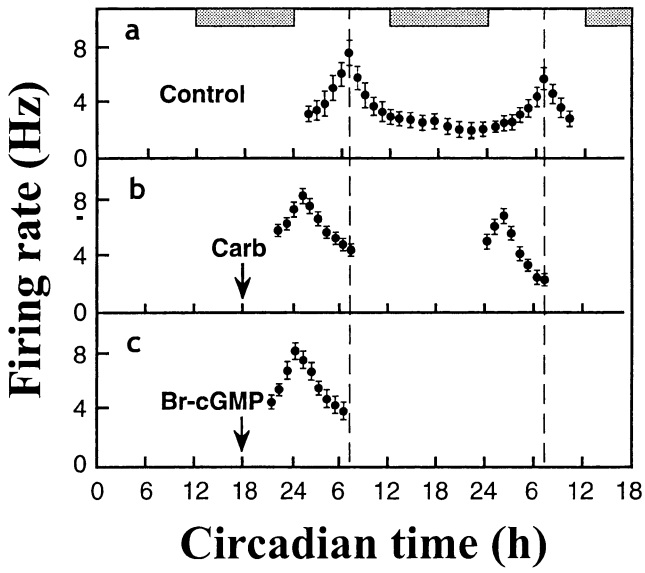

Fig. 1. The circadian rhythm of spontaneous neuronal activity (a) in the rat SCN brain slice is advanced by carbachol (Carb) (b) and 8-Br-cGMP (c) applied mid-subjective night (CT 18).

ergic sensitivity is expressed only during the subjective night (Fig. 1b, 2), that portion of the 24-h cycle that matches the dark (night) period in the animal colony.

The pattern of sensitivity of the SCN to carbachol provided clues as to the signaling pathway by which the cholinergic signal is transmitted to the clock. Carbachol induces phase advance across the night with peak sensitivity at circadian time (CT) 18. (CT is a designation for the temporal state of the clock under constant conditions that starts at CT 0 = "lights on" in the donor animal's colony and continues for 24-h.) At CT 18, carbachol administration advances SCN clock state by ~6 h. The amplitude of this maximal shift and the timing of SCN sensitivity to carbachol are completely overlapping with clock sensitivity to membrane-permeable analogs of the intracellular messenger, cGMP (Fig. 1c, 2) [13,14].

When the predictions of these correlative data were probed experimentally, we found that carbachol directly up-regulated the guanylyl cyclase/cGMP/protein kinase G (PKG) pathway in the SCN of rat. At CT 18, carbachol induced a rapid, transient rise in cGMP in SCN tissue at 3 min, as well as enhanced phosphotransferase activity of PKG toward a preferred substrate; the former effect was block by the anti-muscarinic, atropine [14]. Furthermore, LY83583, a guanylyl cyclase inhibitor, blocked the carbachol-induced clock resetting, but not the shift induced by the analog of its product, cGMP, a downstream effector. On the other hand, the selective PKG inhibitor, KT5823, blocked the effects of carbachol and Br-cGMP, both of which act upstream of PKG. This evidence that the cholinergic signal may be mediated by cGMP implicates the odd-numbered muscarinic receptors (M_1, M_3, M_5) in SCN clock regulation. However, it does not identify a single receptor, nor does it exclude participation in the response by even-numbered receptor subtypes [15].

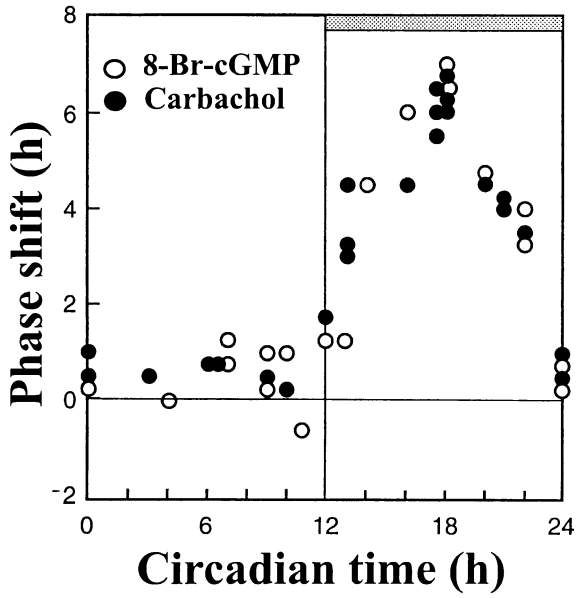

Fig. 2. Carbachol and 8-Br-cGMP induce phase advance of the SCN circadian clock during subjective night, with overlapping periods of sensitivity and amplitudes of response. Each circle is the result of a single experiment evaluating time-of-peak activity, as in Fig. 1b, c.

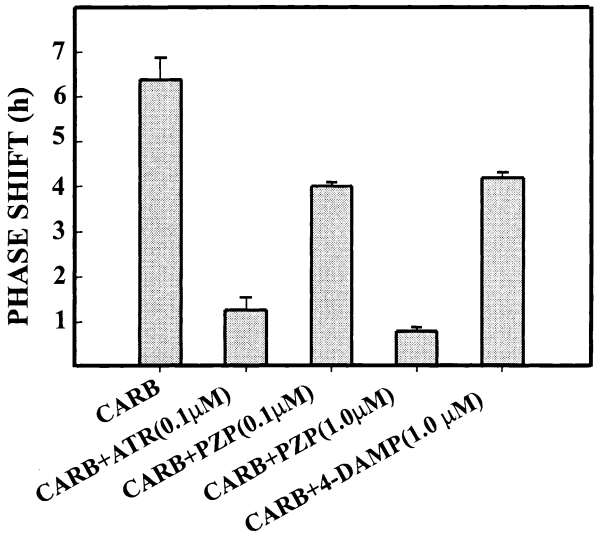

Fig. 3. Relative potency of muscarinic antagonists. atropine (ATR), pirenzepine (PZP) and 4-DAMP, in blocking the effect of carbachol at CT 18 suggests an M_1-like receptor mediated action.

Pharmacological analyses of the cholinergic effects on the SCN clock at CT 18 further characterized receptors mediating clock resetting. Carbachol is a nonselective cholinergic agonist that activates both muscarinic and nicotinic receptors, and both receptors are expressed in SCN [16]. Furthermore, nicotinic effects on the rat SCN have been reported [17,18]. When

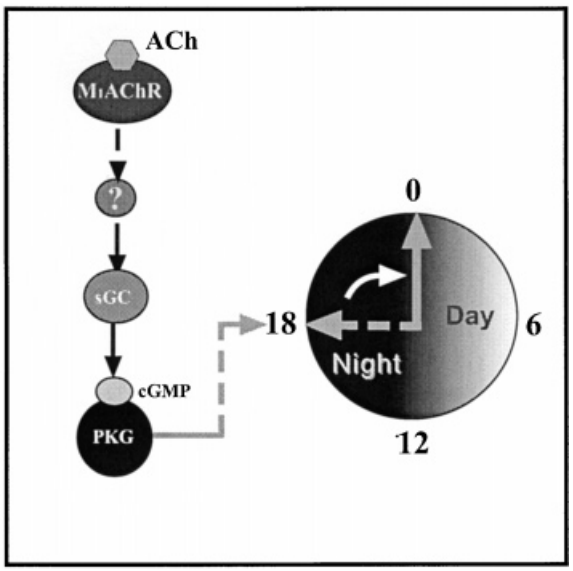

Fig. 4. Model of putative signaling elements that transduce the nocturnal cholinergic signal into phase advance of the SCN circadian clock.

the dose-response relationship among various cholinergics applied by microdrop over a range of concentrations was analyzed for efficacy on phase-resetting, the following relative potency was observed: ACh > McN-A-343 > carbachol = muscarine >>> nicotine [19]. Nicotine was three orders of magnitude less effective than carbachol or muscarine. Nicotinic antagonists, dihydro-β-erythroidine (DHβE) and *d*-tubocurarine, each were ineffective in blocking the carbachol-induced phase advance. However, the effect of carbachol was blocked differentially by muscarinic antagonists, with a relative potency as follows: atropine > pirenzepine > 4,2-(4,4′-diacetoxydiphenylmethyl)pyridine (4-DAMP) (Fig. 3). Relative efficacy of McN-A-343 and of pirenzepine, putatively selective for the M_1 muscarinic subtype, concurred with pharmacological sensitivities in other reports [20,21]. These pharmacological data suggest that the cholinergic receptor mediating SCN clock resetting expresses M_1-like characteristics. A model linking putative elements in the cholinergic pathway signaling phase advance to the SCN clock appears in Fig. 4.

The specificity of these pharmacologic agents for the M_1 receptor subtype has been questioned [15]. However, the development of transgenic mice in which the gene for the M_1 receptor has been lost by homologous recombination (M_1-knock out mice, M_1KO) [22] offers the opportunity to genetically test M_1 receptor contribution to clock regulation. The M_1KO mice develop normally and are generally healthy. Up-regulation of M_2, M_3, or M_4 receptors was not detected in the hippocampus [22]. Deficits of function reported include loss of the muscarinic receptor-dependent M-current K^+ channel activity in sympathetic ganglion neurons, reduced susceptibility to pilocarpine-induced cortical seizure [22], and disabled N- and L-type Ca^{2+}-mediated neurotransmitter release in superior cervical ganglion [23]. Prelimary studies [24] suggest that the M_1KO mouse as well as transgenic mice with other muscarinic receptor deficits offer important models for critically evaluating cholinergic effects on the circadian clock.

Acknowledgments

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