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The effects of cholinergic drugs on rat neocortical high-voltage spindles in ketanserin-treated rats

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Abstract

To investigate the roles of the cholinergic system and 5-HT_2 receptors in the modulation of thalamocortical oscillations, we studied the effects of systemic (s.c.) administration of anticholinesterases (physostigmine, tetrahydroaminoacridine) and muscarinic acetylcholine receptor agonists (pilocarpine, oxotremorine) on spontaneous thalamically generated rhythmic neocortical high-voltage spindles in adult rats pretreated with either saline or ketanserin, a 5-HT_2 receptor antagonist. Ketanserin at 20.0 mg/kg increased the number of high-voltage spindles. In saline-treated rats, tetrahydroaminoacridine 3.0 and 9.0 mg/kg was able to decrease high-voltage spindles, whereas in ketanserin 20.0 mg/kg-treated rats only the highest dose of tetrahydroaminoacridine (9.0 mg/kg) decreased high-voltage spindles. Both doses of physostigmine, 0.12 and 0.36 mg/kg, decreased high-voltage spindles in both saline and ketanserin 20.0 mg/kg-treated rats. Lower doses of tetrahydroaminoacridine (1.0 mg/kg) and physostigmine (0.06 mg/kg) were ineffective in both saline- and ketanserin 20.0 mg/kg-treated rats. Pilocarpine 3.0 mg/kg and oxotremorine 0.1 and 0.9 mg/kg decreased high-voltage spindles in saline-treated rats. However, in rats treated with ketanserin 20.0 mg/kg, only the lower doses of pilocarpine (0.3 and 1.0 mg/kg) and oxotremorine (0.03 mg/kg) were able to decrease the high-voltage spindles. The results suggest that activation of the cholinergic system and activation of 5-HT_2 receptors have additive effects in the suppression of thalamocortical oscillations and related neocortical high-voltage spindles in rats, thus maintaining effective information processing in thalamocortical networks.

Keywords: Anticholinesterase; Muscarinic acetylcholine receptor; 5-HT_2 receptor; Neocortical high-voltage spindle; Thalamocortical oscillation; (Rat)

1. Introduction

The brain cholinergic and serotonergic systems have been shown to interact in the modulation of a number of physiological functions, such as learning and memory, and are thought to be involved in the activation of the cerebral cortex (see, e.g., Sirviö et al., 1994). This can be seen in the appearance of low-voltage fast activity in the neocortex and rhythmical slow activity in the hippocampus (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989), and suppression of neocortical high-voltage spindles generated in thalamocortical networks (Riekkinen et al., 1990; Jäkälä et al., 1995, 1996).

These neocortical high-voltage spindles seem to be attributable to the rhythmic activity of γ-aminobutyric acid (GABA)-containing neurons in the nucleus reticularis of the thalamus which phasically hyperpolarize their thalamocortical target neurons. In the absence of other depolarizing inputs, voltage- and time-dependent rebound Ca^{2+} spikes occur in a phase-locked manner in thalamocortical relay neurons (Steriade and Llinás, 1988; Buzsáki et al., 1990; McCormick, 1992; Steriade et al., 1993). The rhythmical bursts of thalamocortical neurons are transferred also to the cortex, where they induce excitatory post-synaptic potentials in cortical pyramidal neurons, generating the neocortical high-voltage spindles which can be recorded in the cortical electroencephalogram (EEG) (Steriade et al., 1993). Typically, neocortical high-voltage spindles occur only during states of low arousal and low vigilance (drowsiness), being virtually absent during high vigilance states, when the increased activity of the ascending systems (cholinergic, serotonergic, noradrenergic, dopaminergic and histaminergic systems) is thought to suppress rhythmic burst firing in nucleus reticularis neurons by producing depolarization or by blocking hyperpolarization in thalamocortical relay neurons (McCormick, 1992; Steri-

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ade et al., 1993). The number and duration of high-voltage spindles increases with age in those rat strains in which they have been observed (Buzsáki et al., 1990; Sirviö et al., 1989), and importantly, the transfer of information through the thalamus to the cortex and to other structures may be disrupted during thalamic oscillatory activity (McCormick, 1992).

Basal forebrain and brainstem cholinergic neurons have an important role in the desynchronization of neocortical electrical activity (Buzsáki et al., 1988; Steriade and Buzsáki, 1990). The cholinergic projection cells of the basal forebrain and brainstem innervate different thalamic nuclei and cortical areas (Steriade and Buzsáki, 1990; Wainer and Mesulam, 1990). Activation of the cholinergic neurons of the brainstem and basal forebrain inhibits thalamocortically generated high-voltage spindles and neocortical slow waves (Steriade and Buzsáki, 1990). For example, lesions in the cholinergic nucleus basalis induced by infusions with ibotenic or quisqualic acid increase neocortical high-voltage spindles and slow waves (Buzsáki et al., 1988; Riekkinen et al., 1990, 1991, 1992). The importance of the loss of cholinergic neurons to the EEG defect is also supported by recent studies showing that an anticholinesterase drug, tetrahydroaminoacridine, which prevents the breakdown of acetylcholine, alleviates the cortical EEG defect seen after cholinergic lesion (Riekkinen et al., 1991). Furthermore, in aged rats, the increase in high-voltage spindles correlates with the decreased number of cells in the nucleus basalis that are choline acetyltransferase-positive, and a muscarinic acetylcholine receptor agonist, pilocarpine, suppresses high-voltage spindles (Riekkinen et al., 1992). Nucleus basalis projections may suppress rhythmic thalamocortical oscillations in the pacemaker zone, reticular nucleus (Levey et al., 1987; Steriade and Buzsáki, 1990) and suppress neocortical slow waves (Steriade and Buzsáki, 1990). The brainstem projections to the thalamic relay nuclei may regulate the depolarization of glutaminergic thalamocortical projection cells and maintain the thalamus in a transfer mode of action (McCormick, 1989, 1990, 1992; Steriade and Buzsáki, 1990; Steriade et al., 1993). Furthermore, acetylcholine released from ascending brainstem cholinergic neurons may prevent the generation of spindle activity by the GABAergic reticular nucleus (Steriade and Buzsáki, 1990; Steriade et al., 1993).

The loss of cholinergic markers in patients with Alzheimer's disease has also been related to EEG alterations (i.e., slowing of the EEG) which occur during the course of the disease (Soininen et al., 1989; Soininen et al., 1992). It should also be noted that other subcortical modulatory systems, such as the serotonergic system, are adversely affected by aging and Alzheimer's disease (Allen et al., 1983; Mann and Yates, 1983; Yamato and Hirano, 1985; Reinikainen et al., 1990). In the light of the suggested involvement of the brainstem serotonergic projections in neocortical activation (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989; Sirviö et al., 1994), there is the possibility that a senescence-associated serotonergic deficit is an additional factor in the EEG pathology characteristic of aging and Alzheimer's disease (Baskys et al., 1987; Soininen et al., 1989, 1992). Indeed, anatomical studies have shown that the thalamic nuclei are also innervated by serotonergic fibers (Steinbusch, 1981; Jacobs and Azmitia, 1992) that can regulate thalamic functioning, and both in vitro (Pape and McCormick, 1989; McCormick and Wang, 1991; McCormick, 1992) and in vivo (Jäkälä et al., 1995, 1996) electrophysiological studies have shown that activation of 5-HT_2 receptors may suppress thalamocortical oscillations.

The present study was designed to further characterize the roles of the cholinergic system and 5-HT_2 receptors in the modulation of rat thalamocortical oscillations, and to study whether 5-HT_2 receptor blockade modulates the facilitation of thalamocortical arousal (as measured by suppression of neocortical high-voltage spindles) produced by cholinergic stimulation. Therefore, we studied the effects of systemic injections of two anticholinesterase agents, tetrahydroaminoacridine and physostigmine, and two muscarinic acetylcholine receptor agonists, pilocarpine (a mixed muscarinic M_1/M_2 receptor agonist) and oxotremorine (predominantly a muscarinic M_2 receptor agonist), on their own and combined with a 5-HT_2 receptor antagonist, ketanserin, on neocortical high-voltage spindle activity in adult rats.

2. Materials and methods

2.1. Animals

Male Han:Wistar rats (6 months of age at the beginning of electrophysiological recordings; $n = 12$ in the first group and $n = 14$ in the second group) were used in the present study. The rats were singly housed in a controlled environment (National Animal Center, Kuopio, Finland) (temperature 20°C, lights on 07.00–19.00) with water and food available ad libitum. The study design was approved by the Local Ethics Committee.

2.2. Drugs

The selection of drug doses was based on previous electrophysiological tests (Riekkinen et al., 1993b; Jäkälä et al., 1995). Ketanserin tartrate (a 5-HT_2 receptor antagonist; 20.0 mg/kg, s.c., 5.0 ml/kg), tetrahydroaminoacridine (a cholinesterase inhibitor; 1.0, 3.0 and 9.0 mg/kg, i.p., 2.0 ml/kg), (−)-physostigmine sulfate (a cholinesterase inhibitor; 0.06, 0.12 and 0.36 mg/kg, i.p., 2.0 ml/kg), pilocarpine hydrochloride (a mixed muscarinic M_1/M_2 receptor agonist; 0.3, 1.0 and 3.0 mg/kg, i.p., 2.0 ml/kg) and oxotremorine sesquifumarate (predominantly a muscarinic M_2 receptor agonist; 0.03, 0.1

and 0.9 mg/kg, i.p., 2.0 ml/kg), all from Research Biochemicals (Natick, MA, USA), were dissolved in saline and injected 30 min before (except tetrahydroaminoacridine 90 min before) recording neocortical high-voltage spindles. Before being diluted with saline, ketanserin had to be dissolved with a few drops of glacial acetic acid (final pH 6–7).

2.3. Surgery

The animals were anesthetized with Equithesin (3.0 ml/kg, i.p.) and placed in a stereotaxic frame with the incisor bar set at −3.3 mm and the bregma and lambda in the horizontal plane. Active recording electrodes (stainless steel screws 0.5 mm in diameter) were placed symmetrically on both sides above the frontal cortex ($A = 1.0$ mm and $L = \pm 2.0$ mm relative to the bregma). Ground and indifferent electrodes were placed in the midline above the cerebellum and nasal bone, respectively. The screw electrodes and connecting female pins were embedded in dental acrylic. A two week recovery period after implantation of the recording electrodes was allowed before recordings were started.

2.4. High-voltage spindle recordings

Before recordings were made, rats were twice placed in the recording cages for 10 min to habituate them to the recording environment. To ascertain that the high-voltage spindle levels would be as constant as possible, five 20-min cumulative waking–immobility (eyes open, head held up) baseline recordings without any drug injections and one baseline recording with saline injections were made before the experiments. No differences in high-voltage spindle total duration were observed between the fourth and the fifth baseline recording sessions (data not shown). To control for circadian variations or arousal level, recordings after the test drug injections were made at the same time of day for individual animals. Recordings were made between 09.00–15.00 h. During the recordings, the rats were allowed to move freely in the recording cages. The alertness and wakefulness of the rats were constantly monitored by the experimenter during the recordings, and if necessary the rats were aroused by gently touching the vibrissae. High-voltage spindle activity of four rats was recorded simultaneously and analyzed automatically. The IBM-compatible software separated high-voltage spindles from background EEG and calculated the number (incidence), mean duration and total duration (incidence × mean duration) of high-voltage spindles from the right active recording electrode during a 20-min cumulative waking-immobility period. The EEG epoch was considered as a high-voltage spindle if it met the following criteria: (1) the amplitude of EEG was more than twice that of the background EEG (threshold), (2) the duration of each epoch which exceeded the threshold was more than 0.5 s, (3) the frequency of the EEG exceeding the threshold was 6–12 Hz (in previous studies in our laboratory high-voltage spindle activity has been observed within these frequency limits in male Han:Wistar rats: Riekkinen et al., 1990, 1991, 1992, 1993a,b,1995; Jäkälä et al., 1995, 1996), (4) the time between two separate spindles had to be at least 0.5 s (if the time between two spindles was less than 0.5 s, it was considered as one high-voltage spindle) and (5) no movement registered by the magnetic coil binding on the head except vibrissal or head tremor was allowed 1 s before or during each high-voltage spindle epoch. A typical neocortical high-voltage spindle is shown in Fig. 1.

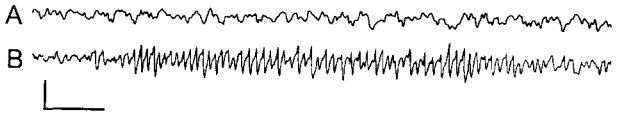

Fig. 1. Example of non-spindling EEG (A) and a typical neocortical high-voltage spindle episode (B) recorded from the right active recording electrode above the frontal cortex ($A = 1.0$ mm and $L = 2.0$ mm relative to the bregma) during a period of relaxed behavioral waking-immobility (eyes open, head held up). The amplitude scale (vertical bar) indicates 300 μV. The time scale (horizontal bar) indicates 1.0 s.

After the baseline recordings were made, the drug effects were tested. The experimental groups and recording schedule are shown in Table 1. In the first group of adult rats the effects of saline (i.p.) + saline (s.c.) and different doses of tetrahydroaminoacridine (1.0, 3.0 and 9.0 mg/kg) (i.p.) + saline (s.c.) were tested in a counterbalanced order every third day. After a one-week wash-out period, the effects of saline (i.p.) + saline (s.c.) and different doses of physostigmine (0.06, 0.12 and 0.36 mg/kg) (i.p.) + saline (s.c.) were tested in a counterbalanced order every third day. Then, after a ten-day wash-out period, the effects of saline (s.c.) + saline (i.p.) and ketanserin 20.0 mg/kg (s.c.) combined with saline (i.p.) or different doses of tetrahydroaminoacridine (1.0, 3.0 and 9.0 mg/kg) (i.p.) were tested in a counterbalanced order every third day. There was then a further two-week wash-out period. During this period, one rat lost its EEG electrode–dental acrylic connection, and so this rat was excluded from further recordings. The effects of saline (s.c.) + saline (i.p.) and ketanserin 20.0 mg/kg (s.c.) combined with saline (i.p.) or different doses of physostigime (0.06, 0.12 or 0.36 mg/kg) (i.p.) were subsequently tested in a counterbalanced order every third day.

In the second group of adult rats, at first the effects of saline (i.p.) + saline (s.c.) and different doses of pilocarpine (0.3, 1.0 and 3.0 mg/kg) (i.p.) + saline (s.c.) were tested in a counterbalanced order every third day. After a one-week wash-out period, the effects of saline (i.p.) + saline (s.c.) and different doses of oxotremorine (0.03, 0.1 and 0.9 mg/kg) (i.p.) + saline (s.c.) were tested in a counterbalanced order every third day. During these recordings, two rats lost their EEG electrode-dental acrylic connections, and these rats were excluded from further

Table 1
Experimental groups and drug treatments used in the present study. There was a 2-day recovery period between the drug recordings, which were made in a counterbalanced order in both groups

Treatment group I ($n = 12$)	Treatment group II ($n = 14$)
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
THA 1.0 (i.p.) + sal (s.c.)	pilo 0.3 (i.p.) + sal (s.c.)
THA 3.0 (i.p.) + sal (s.c.)	pilo 1.0 (i.p.) + sal (s.c.)
THA 9.0 (i.p.) + sal (s.c.)	pilo 3.0 (i.p.) + sal (s.c.)
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
A one-week wash-out period	A one-week wash-out period
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
Physo 0.06 (i.p.) + sal (s.c.)	oxo 0.03 (i.p.) + sal (s.c.)
Physo 0.12 (i.p.) + sal (s.c.)	oxo 0.1 (i.p.) + sal (s.c.)
Physo 0.36 (i.p.) + sal (s.c.)	oxo 0.9 (i.p.) + sal (s.c.)
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
A ten-day wash-out period	A ten-day wash-out period
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
Sal (i.p.) + ket 20.0 (s.c.)	sal (i.p.) + ket 20.0 (s.c.)
THA 1.0 (i.p.) + ket 20.0 (s.c.)	pilo 0.3 (i.p.) + ket 20.0 (s.c.)
THA 3.0 (i.p.) + ket 20.0 (s.c.)	pilo 1.0 (i.p.) + ket 20.0 (s.c.)
THA 9.0 (i.p.) + ket 20.0 (s.c.)	pilo 3.0 (i.p.) + ket 20.0 (s.c.)
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
A two-week wash-out period	A two-week wash-out period
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)
Sal (i.p.) + ket 20.0 (s.c.)	sal (i.p.) + ket 20.0 (s.c.)
Physo 0.06 (i.p.) + ket 20.0 (s.c.)	oxo 0.03 (i.p.) + ket 20.0 (s.c.)
Physo 0.12 (i.p.) + ket 20.0 (s.c.)	oxo 0.1 (i.p.) + ket 20.0 (s.c.)
Physo 0.36 (i.p.) + ket 20.0 (s.c.)	oxo 0.9 (i.p.) + ket 20.0 (s.c.)
Sal (i.p.) + sal (s.c.)	sal (i.p.) + sal (s.c.)

Numbers in parentheses indicate the size of groups. Doses are expressed as mg/kg. Abbreviations: physo = physostigmine; oxo = oxotremorine; pilo = pilocarpine; sal = saline; THA = tetrahydroaminoacridine.

recordings and statistical analyses. Then, after a ten-day wash-out period, the effects of saline (s.c.) + saline (i.p.) and ketanserin 20.0 mg/kg (s.c.) combined with saline (i.p.) or different doses of pilocarpine (0.3, 1.0 and 3.0 mg/kg) (i.p.) were tested in a counterbalanced order every third day. There was then a further two-week wash-out period. During this period, one rat lost its EEG electrode-dental acrylic connection, and the animal was excluded from further recordings. The effects of saline (s.c.) + saline (i.p.) and ketanserin 20.0 mg/kg (s.c.) combined with saline (i.p.) or different doses of oxotremorine (0.03, 0.1 and 0.9 mg/kg) (i.p.) were subsequently tested in a counterbalanced order every third day.

2.5. Statistics

The multivariable analysis of variance (MANOVA) was used to analyse the overall drug treatment effects on neocortical high-voltage spindles. Post-hoc Wilcoxon signed rank test was used to analyse the differences between various drug doses. High-voltage spindle incidence, mean duration and total duration (= incidence × mean duration), as well as total recording time (i.e., the total recording time needed to achieve a 20-min period of behavioral waking-immobility related EEG; movement periods were automatically excluded by the magnet coil movement sensor binding on the rat's head) were analyzed separately for each of the drugs. The changes seen in rat neocortical high-voltage spindle activity were mainly due to changes in high-voltage spindle incidence and total duration, so that when high-voltage spindle incidence was decreased by the drug treatment, the high-voltage spindle total duration was correspondingly decreased; i.e. when a neocortical high-voltage spindle appeared, its mean duration was rather constant. Therefore, only the results of the drug effects on high-voltage spindle total duration are shown. Furthermore, the total recording times, which provide indirect information about the behavioral/motor activity of the rats after drug treatments, are reported. $P < 0.05$ was accepted as significant.

3. Results

3.1. EEG measurements (high-voltage spindle total duration and total recording time)

3.1.1. Group I

3.1.1.1. Tetrahydroaminoacridine (i.p.) in saline (s.c.)-treated rats (Fig. 2, high-voltage spindle total duration; Fig. 6a, total recording time). There was a significant drug treatment effect of tetrahydroaminoacridine on high-voltage spindle total duration ($F(3,33) = 3.46$, $P < 0.05$). Tetrahydroaminoacridine 1.0 mg/kg had no effect ($Z(4, 7) = -1.29$, $P > 0.1$), whereas 3.0 mg/kg slightly ($Z(6,2) = -1.96$, $P = 0.05$) and 9.0 mg/kg significantly ($Z(10,0) = -2.80$, $P < 0.01$) decreased high-voltage spindles versus saline. Tetrahydroaminoacridine 3.0 mg/kg was more effective in decreasing high-voltage spindles than 1.0 mg/kg ($Z(7,3) = -1.99$, $P < 0.05$), and tetrahydroaminoacridine 9.0 mg/kg was more effective than 1.0 mg/kg ($Z(10,0) = -2.80$, $P < 0.01$) or 3.0 mg/kg ($Z(10,0) = -2.80$, $P < 0.01$).

Tetrahydroaminoacridine treatment did not significantly affect the total recording time ($F(3,33) = 1.42$, $P > 0.1$).

3.1.1.2. Tetrahydroaminoacridine (i.p.) in ketanserin (s.c.)-treated rats (Fig. 2, high-voltage spindle total duration; Fig. 6a, total recording time). A significant drug treatment effect on high-voltage spindle total duration was observed ($F(4,44) = 5.34$, $P = 0.001$). Ketanserin 20.0 mg/kg + saline significantly increased high-voltage spindle total duration versus saline + saline ($Z(3,9) = -2.20$, $P < 0.05$). Ketanserin 20.0 mg/kg + tetrahydroaminoacridine 1.0 mg/kg or ketanserin 20.0 mg/kg + tetrahydroaminoacridine 3.0 mg/kg had no effect versus saline + saline ($Z(5,7) = -1.10$, $P > 0.1$ and

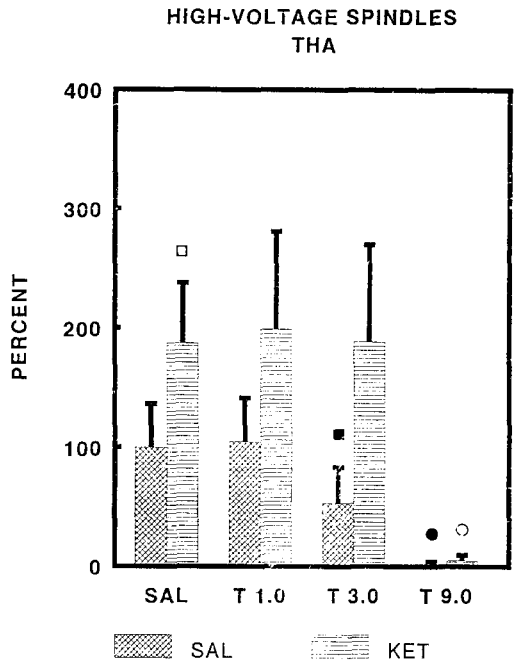

Fig. 2. Effects of systemic administration of an anti-cholinesterase agent, tetrahydroaminoacridine (i.p. 2.0 ml/kg, 90 min pretest), when combined with saline or a 5-HT_2 receptor antagonist, ketanserin (s.c. 5.0 ml/kg, 30 min pretest), on the total duration (incidence × mean duration) of neocortical high-voltage spindles in adult (6–10 months of age, $n = 12$) rats recorded during a 20-min period of cumulative behavioral waking-immobility. The high-voltage spindle recordings were made every third day in a counterbalanced order. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL = saline; T = tetrahydroaminoacridine (doses mg/kg); KET = ketanserin 20.0 mg/kg. The two highest doses of tetrahydroaminoacridine (3.0 and 9.0 mg/kg) decreased high-voltage spindle total duration in saline-treated rats ((■) $P = 0.05$ and (●) $P < 0.01$ versus saline). Tetrahydroaminoacridine 9.0 mg/kg was more effective in suppressing high-voltage spindles than tetrahydroaminoacridine 1.0 mg/kg or 3.0 mg/kg, and 3.0 mg was more effective than 1.0 mg/kg. Ketanserin 20.0 mg/kg alone increased high-voltage spindles ((□) $P < 0.05$ versus saline) and blocked the decrease in high-voltage spindles produced by a moderate dose of tetrahydroaminoacridine (3.0 mg/kg), but not that produced by a high dose of tetrahydroaminoacridine (9.0 mg/kg) ((○) $P < 0.01$ versus ketanserin 20.0 mg/kg + saline).

$Z(6,6) = -0.47$, $P > 0.1$, respectively) or ketanserin 20.0 mg/kg + saline ($Z(8,4) = -0.78$, $P > 0.1$ and $Z(8,4) = -0.70$, $P > 0.1$, respectively). Furthermore, there was no difference between ketanserin 20.0 mg/kg + tetrahydroaminoacridine 1.0 mg/kg and ketanserin 20.0 mg/kg + tetrahydroaminoacridine 3.0 mg/kg ($Z(6,6) = -0.20$, $P > 0.1$). However, tetrahydroaminoacridine 9.0 mg/kg was still capable of effectively suppressing high – voltage spindles when combined with ketanserin 20.0 mg/kg ($Z(11,0) = -2.93$, $P < 0.01$ versus saline + saline; $Z(12,0) = -3.06$, $P < 0.005$ versus ketanserin 20.0 mg/kg + saline; $Z(11,0) = -2.93$, $P < 0.005$ versus ketanserin 20.0 mg/kg + tetrahydroaminoacridine 1.0 mg/kg; $Z(10,1) = -2.76$, $P < 0.01$ versus ketanserin 20.0 mg/kg + tetrahydroaminoacridine 3.0 mg/kg).

There was no significant drug treatment effect on total recording time ($F(4,44) = 1.78$, $P > 0.1$).

3.1.1.3. Physostigmine (i.p.) in saline (s.c.)-treated rats (Fig. 3, high-voltage spindle total duration; Fig. 6b, total recording time). There was a significant drug treatment effect of physostigmine on high-voltage spindle total duration ($F(3,33) = 7.03$, $P = 0.001$). Physostigime 0.06 mg/kg had no effect ($Z(4,6) = -1.38$, $P > 0.1$), whereas 0.12 mg/kg ($Z(2,8) = -2.50$, $P < 0.02$) and 0.36 mg/kg ($Z(0,10) = -2.80$, $P < 0.01$) significantly decreased high-voltage spindles versus saline. Physostigmine 0.12 mg/kg was more effective in decreasing high-voltage spindles than 0.06 mg/kg ($Z(8,2) = -2.45$, $P < 0.02$), and physostigmine 0.36 mg/kg was more effective than 0.06 mg/kg ($Z(10,0) = -2.80$, $P < 0.01$) or 0.12 mg/kg ($Z(6,0) = -2.20$, $P < 0.05$).

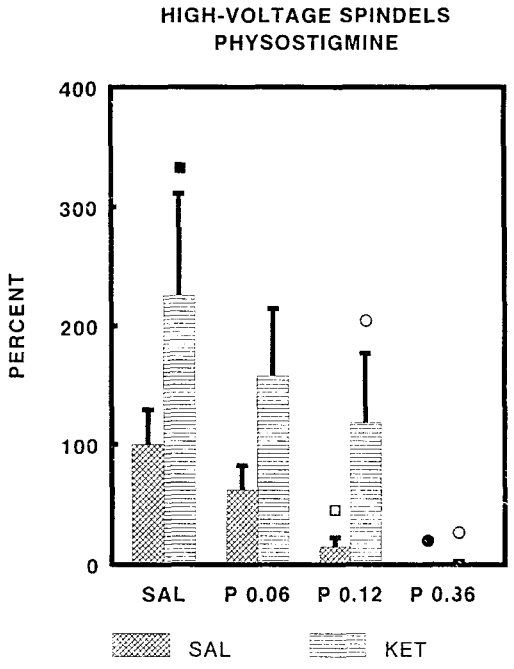

Fig. 3. Effects of systemic administration of an anti-cholinesterase agent, physostigmine (i.p. 2.0 ml/kg, 30 min pretest), when combined with saline or a 5-HT_2 receptor antagonist, ketanserin (s.c. 5.0 ml/kg, 30 min pretest), on the total duration (incidence × mean duration) of neocortical high-voltage spindles in adult (6–10 months of age, $n = 12$) rats recorded during a 20-min period of cumulative behavioral waking-immobility. The high-voltage spindle recordings were made every third day in a counterbalanced order. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL = saline; P = physostigmine (doses mg/kg); KET = ketanserin 20.0 mg/kg. Physostigmine at the two highest doses (0.12 and 0.36 mg/kg) suppressed high-voltage spindles in saline-treated rats ((□) $P < 0.05$ and (●) $P < 0.01$ versus saline), and at the highest dose (0.36 mg/kg) was more effective than at the other doses. Ketanserin 20.0 mg/kg alone increased high-voltage spindles ((■) $P < 0.05$ versus saline), and in ketanserin 20.0 mg/kg-treated rats the two highest doses of physostigmine (0.12 and 0.36 mg/kg) were still capable of decreasing high-voltage spindles ((○) $P < 0.02$ versus ketanserin 20.0 mg/kg + saline).

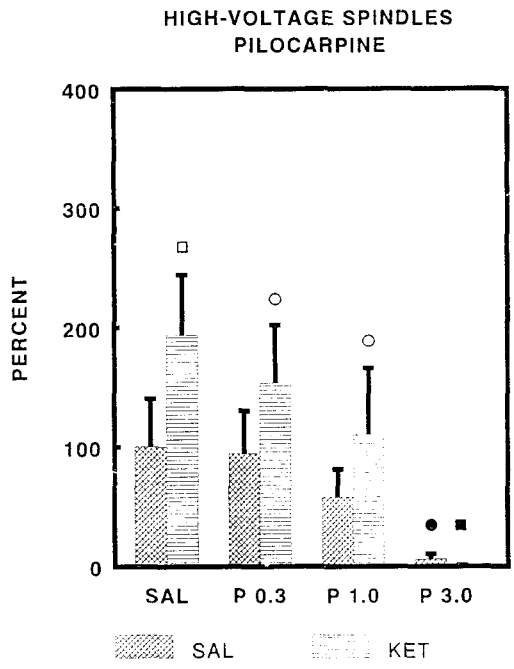

Fig. 4. Effects of systemic administration of a mixed M_1/M_2 muscarinic receptor agonist, pilocarpine (i.p. 2.0 ml/kg 30 min pretest), when combined with saline or a 5-HT_2 receptor antagonist, ketanserin (s.c. 5.0 ml/kg, 30 min pretest), on the total duration (incidence × mean duration) of neocortical high-voltage spindles in adult (6–10 months of age, $n = 14$) rats recorded during a 20-min period of cumulative behavioral waking-immobility. The high-voltage spindle recordings were made every third day in a counterbalanced order. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL = saline; P = pilocarpine (doses mg/kg); KET = ketanserin 20.0 mg/kg. Only the highest dose of pilocarpine (3.0 mg/kg) suppressed high-voltage spindles in saline-treated rats ((●) $P < 0.01$ versus saline). Ketanserin 20.0 mg/kg alone increased high-voltage spindles ((□) $P < 0.05$ versus saline). However, in ketanserin 20.0 mg/kg-treated rats lower doses of pilocarpine (0.3 and 1.0 mg/kg) were able to decrease high-voltage spindles ((○) $P < 0.02$ and (■) $P < 0.01$ versus ketanserin 20.0 mg/kg + saline).

Physostigmine treatment also significantly affected the total recording time ($F(3,33) = 5.10$, $P < 0.01$). All the doses of physostigmine increased the total recording time (physostigmine 0.06 mg/kg: $Z(0,8) = -2.52$, $P < 0.02$; physostigmine 0.12 mg/kg: $Z(1,8) = -2.49$, $P < 0.02$; physostigmine 0.36 mg/kg: $Z(2,8) = -1.99$, $P < 0.05$ versus saline, respectively). There was no difference between physostigmine 0.06 and 0.12 mg/kg doses ($Z(3,7) = -1.94$, $P > 0.05$), physostigmine 0.06 and 0.36 mg/kg doses ($Z(2,7) = -1.42$, $P > 0.1$) or physostigmine 0.12 and 0.36 mg/kg doses ($Z(2,8) = -1.22$, $P > 0.1$).

3.1.1.4. Physostigmine (i.p.) in ketanserin (s.c.)-treated rats (Fig. 3, high-voltage spindle total duration; Fig. 6b, total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(4,40) = 5.80$, $P = 0.001$). Ketanserin 20.0 mg/kg + saline and ketanserin 20.0 mg/kg + physostigmine 0.06 mg/kg significantly increased high-voltage spindle total duration versus saline + saline ($Z(7,3) = -1.99$, $P < 0.05$ and $Z(2,8) = -2.50$, $P < 0.02$, respectively), and these two drug combinations did not differ from each other ($Z(5,4) = -1.48$, $P > 0.1$). Ketanserin 20.0 mg/kg + physostigmine 0.12 mg/kg had no effect versus saline + saline ($Z(7,4) = -0.09$, $P > 0.1$), decreased high-voltage spindles versus ketanserin 20.0 mg/kg + saline ($Z(8,1) = -2.42$, $P < 0.02$), and did not differ from ketanserin 20.0 mg/kg + physostigmine 0.06 mg/kg ($Z(7,3) = -1.27$, $P > 0.1$). However, physostigmine 0.36 mg/kg was still capable of virtually completely suppressing high – voltage spindles when combined with ketanserin 20.0 mg/kg ($Z(10,1) = -2.85$, $P < 0.01$ versus saline + saline; $Z(8,1) = -2.57$, $P < 0.02$ versus ketanserin 20.0 mg/kg + saline; $Z(10,0)$

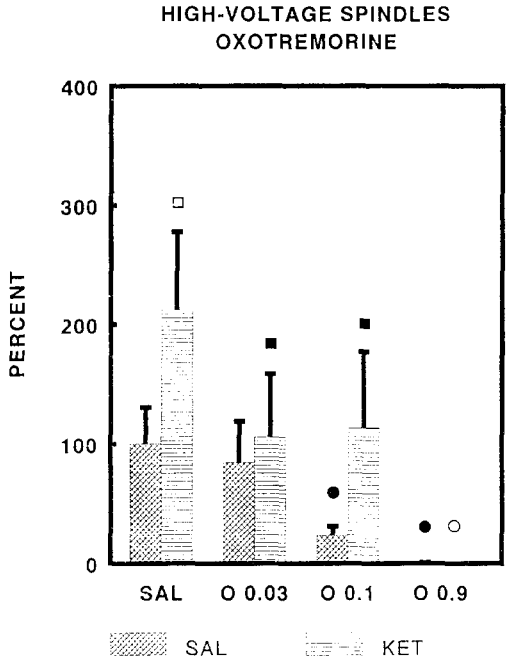

Fig. 5. Effects of systemic administration of a predominantly M_2 muscarinic receptor agonist, oxotremorine (i.p. 2.0 ml/kg 30 min pretest), when combined with saline or a 5-HT_2 receptor antagonist, ketanserin (s.c. 5.0 ml/kg, 30 min pretest), on the total duration (incidence × mean duration) of neocortical high-voltage spindles in adult (6–10 months of age, $n = 14$) rats recorded during a 20-min period of cumulative behavioral waking-immobility. The high-voltage spindle recordings were made every third day in a counterbalanced order. Values represent % group means ± S.E.M. of control (saline-treated) values (100%). Abbreviations: SAL = saline; O = oxotremorine (doses mg/kg); KET = ketanserin 20.0 mg/kg. Oxotremorine at the two highest doses (0.1 and 0.9 mg/kg) suppressed high-voltage spindles in saline-treated rats ((●) $P < 0.01$ versus saline), and at the highest dose (0.9 mg/kg) was more effective than at the other doses. Ketanserin 20.0 mg/kg alone increased high-voltage spindles ((□) $P < 0.01$ versus saline). In ketanserin 20.0 mg/kg-treated rats oxotremorine at a lower dose (0.03 mg/kg) decreased high-voltage spindles ((■) $P < 0.01$ and (○) $P < 0.005$ versus ketanserin 20.0 mg/kg + saline).

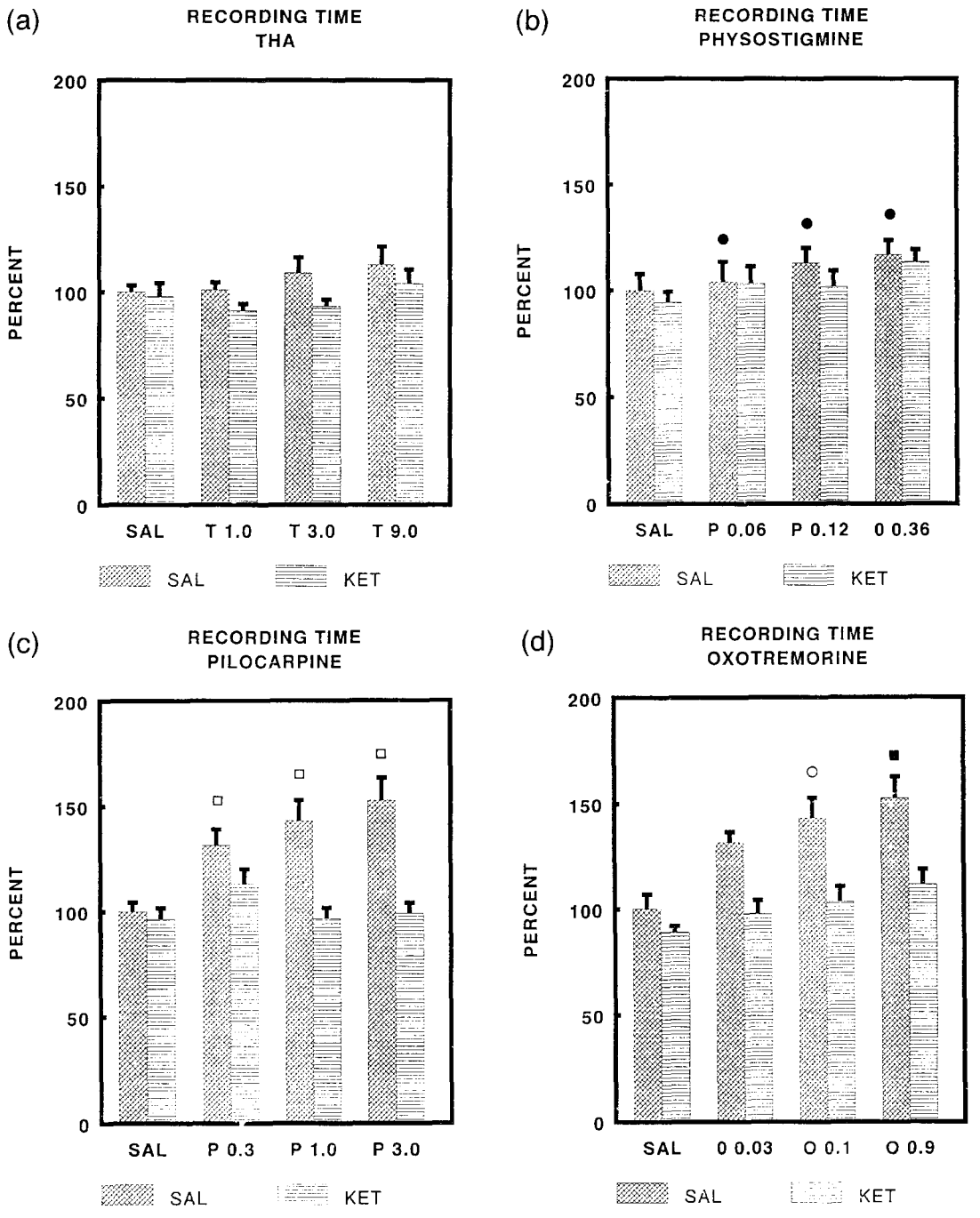

Fig. 6. The effects of the drugs used in the present study on the total recording times (the total time needed to achieve a cumulative 20-min period of relaxed quiet behavioral waking-immobility = immobility + movement periods). Movement periods were automatically registered and excluded from the high-voltage spindle data analysis by a magnet-coil movement-sensor detector of the EEG cables which was bound to the rat's head. Recordings were made every third day in 6–10 month-old male Han:Wistar rats. Values (min) represent group means ± S.E.M. For abbreviations see Fig. 2Fig. 3Fig. 4Fig. 5. (a) Tetrahydroaminoacridine and ketanserin had no effect on total recording times. (b) All the doses of physostigmine increased total recording times in saline-treated rats, and there was no difference between the doses ((●) $P < 0.02$ versus saline). Ketanserin 20.0 mg/kg alone had no effect, but antagonized the increase in total recording times produced by physostigmine. (c) Pilocarpine at all doses increased total recording time in saline-treated rats ((□) $P < 0.005$ versus saline). However, ketanserin 20.0 mg/kg, although alone having no effect, blocked the increase in total recording time produced by pilocarpine. (d): Oxotremorine at 0.1 mg/kg slightly and at 0.9 mg/kg significantly increased total recording time in saline-treated rats ((○) $P = 0.05$ and (■) $P < 0.02$ versus saline). Ketanserin 20.0 mg/kg, although alone having no effect, blocked the increase in total recording time produced by oxotremorine.

$= -2.80$, $P < 0.01$ versus ketanserin 20.0 mg/kg + physostigmine 0.06 mg/kg; $Z(8,1) = -2.43$, $P < 0.02$ versus ketanserin 20.0 mg/kg + physostigmine 0.12 mg/kg).

In the total recording time analyses, no significant drug treatment effects were observed ($F(4,40) = 1.95$, $P > 0.1$).

3.1.2. Group II

3.1.2.1. Pilocarpine (i.p.) in saline (s.c.)-treated rats (Fig. 4, high-voltage spindle total duration; Fig. 6c, total recording time). There was a significant drug treatment effect of pilocarpine on high-voltage spindle total duration ($F(3,39) = 5.46$, $P < 0.005$). Pilocarpine 0.3 mg/kg or 1.0 mg/kg had no effect ($Z(4,7) = -0.09$, $P > 0.1$ and $Z(6,4) = -1.38$, $P > 0.1$, respectively), whereas pilocarpine 3.0 mg/kg suppressed high − voltage spindles ($Z(10,0) = -2.80$, $P < 0.01$) versus saline. Pilocarpine 3.0 mg/kg was more effective in decreasing high-voltage spindles than 0.3 mg/kg ($Z(12,0) = -3.06$, $P < 0.005$), or 1.0 mg/kg ($Z(12,0) = -3.06$, $P < 0.005$), and pilocarpine 1.0 mg/kg decreased high-voltage spindles more than pilocarpine 0.3 mg/kg did ($Z(9,2) = -2.22$, $P < 0.05$).

There was also a significant drug treatment effect of pilocarpine on the total recording time ($F(3,39) = 20.28$, $P < 0.001$). All the doses of pilocarpine increased the total recording time versus saline (0.3 mg/kg: $Z(0,14) = -3.30$, $P = 0.001$; 1.0 mg/kg: $Z(0,14) = -3.30$, $P = 0.001$; 3.0 mg/kg: $Z(0,14) = -3.30$, $P = 0.001$, respectively). Pilocarpine 1.0 mg/kg slightly increased total recording times versus 0.3 mg/kg ($Z(3,10) = -1.96$, $P = 0.05$), and 3.0 mg/kg increased the total recording time versus 0.3 mg/kg ($Z(5,8) = -2.13$, $P < 0.05$), but not versus 1.0 mg/kg ($Z(6,6) = -0.78$, $P > 0.1$).

3.1.2.2. Pilocarpine (i.p.) in ketanserin (s.c.)-treated rats (Fig. 4, high-voltage spindle total duration; Fig. 6c, total recording time). A significant drug treatment effect on high − voltage spindle total duration was observed ($F(4,44) = 8.32$, $P < 0.001$). Ketanserin 20.0 mg/kg + saline and ketanserin 20.0 mg/kg + pilocarpine 0.3 mg/kg significantly increased high-voltage spindle total duration versus saline + saline ($Z(1,11) = -2.75$, $P < 0.01$ and $Z(3,9) = -2.11$, $P < 0.05$, respectively). However, when combined with ketanserin 20.0 mg/kg, pilocarpine 0.3 mg/kg was able to decrease high-voltage spindle total duration when compared to ketanserin 20.0 mg/kg + saline ($Z(10,2) = -2.43$, $P < 0.02$). Ketanserin 20.0 mg/kg + pilocarpine 1.0 mg/kg had no effect versus saline + saline ($Z(6,6) = -0.16$, $P > 0.1$), but decreased high-voltage spindles when compared to ketanserin 20.0 mg/kg + saline ($Z(10,2) = -2.35$, $P < 0.02$), or to ketanserin 20.0 mg/kg + pilocarpine 0.3 mg/kg ($Z(10,2) = -2.12$, $P < 0.05$). Pilocarpine 3.0 mg/kg was still capable of effectively suppressing high-voltage spindle total duration when combined with ketanserin 20.0 mg/kg ($Z(11,1) = -2.90$, $P < 0.005$ versus saline + saline; $Z(12,0) = -3.06$, $P < 0.005$ versus ketanserin 20.0 mg/kg + saline; $Z(12,0) = -3.06$, $P < 0.005$ versus ketanserin 20.0 mg/kg + pilocarpine 0.3 mg/kg; $Z(11,1) = -2.98$, $P < 0.005$ versus ketanserin 20.0 mg/kg + pilocarpine 1.0 mg/kg).

In the total recording time analyses, no significant drug treatment effect was observed ($F(4,44) = 1.73$, $P > 0.1$).

3.1.2.3. Oxotremorine (i.p.) in saline (s.c.)-treated rat (Fig. 5, high-voltage spindle total duration; Fig. 6d, total recording time). There was a significant drug treatment effect of oxotremorine on high-voltage spindle total duration ($F(3,33) = 7.40$, $P = 0.001$). Oxotremorine 0.03 mg/kg had no effect ($Z(7,5) = -0.67$, $P > 0.1$), whereas 0.1 mg/kg ($Z(12,0) = -3.06$, $P < 0.005$) and 0.9 mg/kg ($Z(12,0) = -3.06$, $P < 0.005$) significantly decreased high-voltage spindles versus saline. Oxotremorine 0.1 mg/kg was more effective in decreasing high-voltage spindles than 0.03 mg/kg was ($Z(11,1) = -2.82$, $P < 0.005$), and oxotremorine 0.9 mg/kg was more effective than 0.03 mg/kg ($Z(12,9) = -3.06$, $P < 0.005$) or 0.1 mg/kg ($Z(10,0) = -2.80$, $P < 0.01$).

Oxotremorine treatment also affected the total recording time ($F(3,33) = 8.65$, $P < 0.001$). Oxotremorine 0.1 mg/kg slightly ($Z(3,8) = -1.96$, $P = 0.05$) and oxotremorine 0.9 mg/kg significantly ($Z(3,9) = -2.39$, $P < 0.02$) increased the total recording time versus saline, whereas 0.03 mg/kg had no effect ($Z(5,6) = -0.22$, $P > 0.1$). Oxotremorine 0.1 mg/kg increased the total recording time versus 0.03 mg/kg ($Z(3,9) = -2.20$, $P < 0.05$), and 0.9 mg/kg increased the total recording time versus 0.03 mg/kg ($Z(1,11) = -2.90$, $P < 0.005$) or 0.1 mg/kg ($Z(2,10) = -2.04$, $P < 0.05$).

3.1.2.4. Oxotremorine (i.p.) in ketanserin (s.c.)-treated rats (Fig. 5, high-voltage spindle total duration; Fig. 6d, total recording time). There was a significant drug treatment effect on high-voltage spindle total duration ($F(4,40) = 6.465.80$, $P < 0.001$). Ketanserin 20.0 mg/kg + saline significantly increased high-voltage spindle total duration versus saline + saline ($Z(1,10) = -2.58$, $P < 0.01$). Ketanserin 20.0 mg/kg + oxotremorine 0.03 mg/kg or ketanserin 20.0 mg/kg + oxotremorine 0.1 mg/kg had no effect versus saline + saline ($Z(6,5) = -0.31$, $P > 0.1$ and $Z(7,3) = -0.66$, $P > 0.1$, respectively), but were capable of decreasing high-voltage spindles when compared to ketanserin 20.0 mg/kg + saline ($Z(10,1) = -2.58$, $P < 0.01$ and $Z(10,1) = -2.58$, $P < 0.01$, respectively). There was no difference between ketanserin 20.0 mg/kg + oxotremorine 0.03 mg/kg and ketanserin 20.0 mg/kg + oxotremorine 0.1 mg/kg ($Z(6,4) = -0.10$, $P > 0.1$). Oxotremorine 0.9 mg/kg still effectively suppressed high − voltage spindles when combined with ketanserin 20.0 mg/kg ($Z(11,0) = -2.93$, $P < 0.005$ versus saline + saline; $Z(11,0) = -2.93$, $P < 0.005$ versus ketanserin 20.0

mg/kg + saline; $Z(10,0) = -2.80$, $P < 0.01$ versus ketanserin 20.0 mg/kg + oxotremorine 0.03 mg/kg; $Z(9,0) = -2.67$, $P < 0.01$ versus ketanserin 20.0 mg/kg + oxotremorine 0.1 mg/kg).

There were no significant drug treatment effects on total recording times ($F(4,40) = 2.32$, $P > 0.05$).

4. Discussion

In line with previous studies (Riekkinen et al., 1991, 1993b; Danober et al., 1993; Jäkälä et al., 1995), the anticholinesterases, tetrahydroaminoacridine and physostigmine, and the muscarinic acetylcholine receptor agonists, pilocarpine (a mixed muscarinic M_1/M_2 receptor agonist) and oxotremorine (predominantly a muscarinic M_2 receptor agonist), effectively decreased neocortical high-voltage spindles in adult rats, and the 5-HT_2 receptor antagonist, ketanserin, significantly increased the number of high-voltage spindles. The main results of the present study were, however, that (A) ketanserin blocked the decrease in high-voltage spindles produced by a moderate dose of tetrahydroaminoacridine, but did not modulate the high-voltage spindle activity suppressing effects of physostigmine, and that (B) both muscarinic acetylcholine receptor agonists, pilocarpine and oxotremorine, decreased high-voltage spindles induced by ketanserin at even lower doses than were required to decrease high-voltage spindles in saline-treated rats.

Tetrahydroaminoacridine and physostigmine are cholinesterase inhibitors which act by preventing the breakdown of acetylcholine, and thus increase the amount of acetylcholine present in synapses. Furthermore, tetrahydroaminoacridine also possesses muscarinic M_1, M_2 (Pearce and Potter, 1988; Flynn and Mash, 1989) and nicotinic (Perry et al., 1988) agonistic activities, and also modulates K^+/Na^+ channels (Scauf and Sattin, 1988). Therefore, the site of action of systemically administered tetrahydroaminoacridine (and physostigmine) in modulating thalamically generated high-voltage spindles may involve both pre- and postsynaptic nicotinic and muscarinic acetylcholine receptors. It is thought that the cholinomimetic effects of tetrahydroaminoacridine are responsible for the suppression of thalamocortical oscillations. This is supported by previous data showing that tetrahydroaminoacridine to some extent could alleviate the increase in high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis (Riekkinen et al., 1991) or by aging-associated atrophy and loss of cholineacetyltransferase-positive cells in the nucleus basalis (Riekkinen et al., 1992). However, it is also possible that the increase in the activity of biogenic amines induced by tetrahydroaminoacridine (Tachiki et al., 1988) may contribute to its ability to suppress high-voltage spindle activity. Indeed, the dose of tetrahydroaminoacridine required to decrease high-voltage spindles is 9-fold higher than that required to inhibit scopolamine-induced slow waves, whereas physostigmine, 'a pure cholinesterase inhibitor', decreases high-voltage spindles and scopolamine-induced slow waves at similar doses (Riekkinen et al., 1993b).

The agonists evaluated in the present study (pilocarpine and oxotremorine) may differ in their muscarinic M_1 versus M_2 receptor selectivity. Both pilocarpine and oxotremorine contract guinea pig bladder, a tissue which contains almost exclusively muscarinic M_2 receptors (Noronha-Blob et al., 1989). Furthermore, both of these compounds decrease the release of acetylcholine in vitro from brain slices and elevate brain acetylcholine levels in vivo (Hadhazy and Szerb, 1977; Szerb et al., 1977; Sethy and Francis, 1988). It has also been shown that these drugs possess low binding affinity for muscarinic M_1 receptors (Potter and Ferrendelli, 1989). Oxotremorine has been proposed to act mainly on muscarinic M_2 receptors, whereas pilocarpine may be a mixed muscarinic M_1 and M_2 receptor agonist (Bräuner-Osborne and Brann, 1996).

Previous electrophysiological studies have shown that the basal forebrain and brainstem cholinergic afferents may regulate neocortical electrical activity via muscarinic acetylcholine receptors (Buzsáki et al., 1988; Riekkinen et al., 1991, 1993b). For example, in rats, systemic administration of low to moderate doses of the muscarinic acetylcholine receptor antagonist, scopolamine, increases the incidence of high-voltage spindles (Riekkinen et al., 1991, 1993b), and spike-and-wave discharges (Danober et al., 1993), which are also thought to reflect the same phenomenon, i.e., thalamocortical oscillations (Micheletti et al., 1987; Danober et al., 1993). Systemic administration of the muscarinic acetylcholine receptor agonists, oxotremorine, pilocarpine and AF-102B (predominantly a muscarinic M_1 receptor agonist), suppresses rat high-voltage spindles in a dose-dependent manner and induces an arousal-like pattern on the cortical EEG (Riekkinen et al., 1993b). Activation of nicotinic acetylcholine receptors may also effectively suppress thalamocortical oscillations, since it has been shown that systemic treatment with nicotine can decrease neocortical high-voltage spindles in adult (Radek, 1993; Riekkinen et al., 1993a; Jäkälä et al., 1996) and aged (Jäkälä et al., 1996) rats, spike-and-wave discharges in adult rats (Danober et al., 1993), and brief spindle episodes in mice (Ryan, 1985). Indeed, activation of presynaptic nicotinic acetylcholine receptors may increase the release of acetylcholine (Beani et al., 1989) in areas important for the modulation of high-voltage spindle activity (Buzsáki et al., 1988, 1990; McCormick, 1990, 1992; Steriade and Buzsáki, 1990). In a previous study, pilocarpine was more potent in suppressing high-voltage spindles in adult rats than was AF102B or oxotremorine (Riekkinen et al., 1993b). However, it is pertinent to note that there are more muscarinic M_2 receptors than muscarinic M_1 receptors in many of the thalamic nuclei which are considered to participate in the generation and modula-

tion of thalamocortical oscillations (Wang et al., 1989; Flynn and Mash, 1993; Wei et al., 1994). Therefore, it may be that both M_1 and M_2 receptors play a role in the modulation of thalamocortical oscillations and related neocortical high-voltage spindles.

The 5-HT_2 receptor antagonist, ketanserin (Leysen et al., 1981), on its own at a relatively high dose (20.0 mg/kg), increased neocortical high-voltage spindle activity, confirming our previous observations (Jäkälä et al., 1995). Previously, lower doses of ketanserin (1.0 and 5.0 mg/kg) had no effect on high-voltage spindle activity in adult rats (Jäkälä et al., 1995) and, therefore, a high dose of ketanserin was chosen for the present study. However, at the dose of ketanserin that was required to increase high-voltage spindles in the present study (20.0 mg/kg), ketanserin may also have non-specific effects, especially binding to α_1-adrenoceptors and histamine (H_1) receptors (Leysen et al., 1981). It cannot be excluded that this partially accounts for the observed increase in high-voltage spindle activity induced by ketanserin, since α_1-adrenoceptors have been shown to play an important role in the modulation of thalamic oscillations and related neocortical high-voltage spindles and spike-and-wave discharges (Micheletti et al., 1987; Buzsáki et al., 1990). In our previous study, ketanserin already at low doses (1.0 or 5.0 mg/kg), doses which on their own had no effect, blocked the decrease in high-voltage spindle activity seen after either systemic (0.5, 1.0 or 2.0 mg/kg) or intrathalamic (ventroposteromedial thalamic area, dose 10 or 50 μg) administration of a specific 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), suggesting that 5-HT_2 receptor antagonism may nonetheless be the mechanism of action of ketanserin in the modulation of thalamocortical oscillations and neocortical high-voltage spindles (Jäkälä et al., 1995). Ketanserin displays some selectivity for 5-HT_{2A} receptors, but also has weak affinity for 5-HT_{2C} receptors (30–50-times lower than its affinity for 5-HT_{2A} receptors) and 5-HT_{2B} receptors (Martin and Humphrey, 1994). DOI is often regarded as a subtype selective 5-HT_{2A} receptor agonist, but it has affinity for 5-HT_{2B} and 5-HT_{2C} receptors as well (Boess and Martin, 1994; Martin and Humphrey, 1994).

In the present study, interesting interactions between the cholinergic system and 5-HT_2 receptors in the modulation of rat thalomocortical oscillations were observed. Firstly, in ketanserin 20 mg/kg-treated rats both muscarinic acetylcholine receptor agonists, pilocarpine and oxotremorine, at low doses (pilocarpine 0.3 mg/kg, oxotremorine 0.03 mg/kg) decreased high-voltage spindles, these doses being lower than those required to decrease 'spontaneous' high-voltage spindles in saline-treated rats (pilocarpine 3.0 mg/kg, oxotremorine 0.1 mg/kg). Secondly, ketanserin prevented the decrease in high-voltage spindle activity produced by a moderate dose of tetrahydroaminoacridine (3.0 mg/kg), but not that produced by physostigmine (0.12 and 0.36 mg/kg) or a high dose of tetrahydroaminoacridine (9.0 mg/kg). According to the results of studies using slicing techniques, both nicotinic and muscarinic acetylcholine receptors as well as 5-HT_2 receptors are involved in the modulation of thalamocortical neurotransmission. In thalamic slice preparations, the fast excitatory response of thalamocortical relay neurons in guinea-pig and cat lateral geniculate nuclei seen after the application of acetylcholine is associated with substantial increases in membrane cationic conductance, and this is mimicked by the application of nicotinic acetylcholine receptor agonists (McCormick, 1990, 1992). This rapid depolarization seen after acetylcholine application is then followed by a slow muscarinic depolarization resulting from the suppression of a resting potassium conductance. Furthermore, application of acetylcholine to GABAergic interneurons in the lateral geniculate nucleus or reticular nucleus results in their inhibition through an increase in membrane potassium conductance, this being mediated by the M_2 subclass of acetylcholine receptors (McCormick, 1990, 1992). In slice preparations from guinea-pig nucleus reticularis neurons and cat perigeniculate nucleus, application of serotonin results in pronounced and prolonged excitation associated with the appearance of single spike activity, this excitatory response to serotonin application being specifically mimicked by 5-HT_2 receptor agonists and blocked by 5-HT_2 receptor antagonists (McCormick and Wang, 1991). Thus, the present results showing that muscarinic acetylcholine receptor agonists, pilocarpine and oxotremorine, effectively decrease high-voltage spindles induced by the 5-HT_2 receptor antagonist, ketanserin, are in line with in vitro results and suggest a common mechanism of action at the thalamic level, i.e., ketanserin may have increased the level of hyperpolarization of thalamocortical relay neurons, and thus the effects of muscarinic acetylcholine receptor agonists (i.e., a decrease in the membrane potential of thalamocortical relay neurons) were seen already at lower doses in ketanserin-treated rats than in saline-treated rats. The finding that ketanserin blocked the decrease in high-voltage spindles elicited by a moderate (but not high) dose of tetrahydroaminoacridine may be related to the aminergic effects of tetrahydroaminoacridine, i.e., the above mentioned aminergic effects of tetrahydroaminoacridine (Tachiki et al., 1988) may, indeed, at least partially account for the ability of this agent to suppress high-voltage spindle activity.

However, the cholinergic drugs and ketanserin may also have acted via an independent site of action to modulate neurophysiological phenomena that regulate high-voltage spindle activity. Anatomical studies have shown that nicotinic and muscarinic acetylcholine and 5-HT_2 receptors are widely distributed in different forebrain structures, such as cortex, basal ganglia and thalamus (Molineaux et al., 1989; Wada et al., 1989; Wainer and Mesulam, 1990; Jacobs and Azmitia, 1992; McCormick, 1992), suggesting that systemically administered cholinergic and 5-HT_2 receptor subtype-specific drugs may act via several brain areas that

regulate thalamocortical oscillations. Therefore, it is possible that the drugs used in the present study acted at different anatomical levels of the thalamocortical circuitry to inhibit high-voltage spindles. However, an equally plausible theory is that the drugs also act at some common brain nuclei, such as reticular or relay nuclei of the thalamus, that could mediate their ability to suppress high-voltage spindle activity. Indeed, as mentioned above, in vitro electrophysiological evidence suggests that activation of nicotinic and muscarinic acetylcholine and 5-HT_2 receptors may decrease hyperpolarization of thalamocortical relay neurons and prevent the activation of Ca^{2+}-mediated spiking and the generation of oscillatory firing (McCormick and Wang, 1991; McCormick, 1992). Further, we have shown that infusion of a 5-HT_2 receptor agonist, DOI, (Jäkälä et al., 1995) or nicotine (Riekkinen et al., 1995) into the ventroposteromedial thalamic area sensory relay nuclei can suppress high-voltage spindles, indicating that activation of nicotinic acetylcholine and 5-HT_2 receptors located in this thalamic area reduces thalamic oscillations, and that peripherally injected cholinergic and 5-HT_2 receptor subtype specific drugs may act via the thalamus to modulate spindle activity.

Partial raphe dorsalis lesions alone do not affect neocortical high-voltage spindle activity, but the lesion aggravates the increase of high-voltage spindle activity induced by partial lesions of the cholinergic nucleus basalis magnocellularis (Riekkinen et al., 1990). Anatomical studies provide further evidence for the sites of interaction between the cholinergic and serotonergic systems in the modulation of thalamocortical oscillations and their related neocortical high-voltage spindles: the basal forebrain area containing cortically and thalamically projecting cholinergic neurons is innervated by the raphe dorsalis, and the brainstem serotonergic cell groups receive cholinergic inputs from the basal forebrain (Zaborsky et al., 1991; Jacobs and Azmitia, 1992). Moreover, the cortical areas and the thalamic reticular nucleus, as well as thalamocortical relay nuclei, receive inputs from basal forebrain and brainstem cholinergic neurons as well from the raphe dorsalis (Steinbusch, 1981; Wainer and Mesulam, 1990; Jacobs and Azmitia, 1992). Therefore, the cholinergic and serotonergic systems could interact at the cell body level, thalamus or cortex. Future studies with local microinfusion of cholinergic and serotonergic drugs should elucidate the sites of these possible interactions.

The recording time data (i.e., the total time needed to achieve a 20-min period of behavioral waking-immobility after drug treatment) indicated that systemic treatment with physostigmine, pilocarpine and oxotremorine increased the motor/behavioral activity of the rats. Furthermore, there was also a trend for tetrahydroaminoacridine to increase recording times, although this did not reach statistical significance. However, in the present study, the effects of these drugs on high-voltage spindles reflect a behavioral waking-immobility state in the animals since a magnetic coil movement-sensor in the EEG cable automatically excluded all the movement-related EEG epochs from the high-voltage spindle recordings. Therefore, recordings are likely to reflect the effects of the drug treatments on neocortical high-voltage spindle activity during quiet waking-immobility behavior itself, and not on behavioral/motor activity as an intermediate variable. Interestingly, ketanserin prevented the increase in behavioral/motor activity produced by the cholinomimetic drugs, as indicated by the decrease in total recording time to control (saline-treated) values. The discharge activity of the raphe serotonergic neurons is closely related to the activity of central motor systems, and it has been suggested that the primary role of serotonergic neurons is to send information to their target neurons with respect to the level of motor activity/behavioral state of the organism (Jacobs and Azmitia, 1992). Furthermore, it has been speculated that serotonergic neurons may act to facilitate motor systems while suppressing sensory systems (Aghajanian and Vandermaelen, 1986). Thus, it could be speculated that in the present study ketanserin blocked the increase in behavioral/motor activity (i.e., the increase in total recording times) induced by cholinomimetic drugs through disfacilitation of motor output systems. In line with this, in rats, 5-HT_2 receptors are found in the basal ganglia and frontoparietal motor cortex with lower numbers being present in the frontoparietal or occipital sensory, striate and auditory cortex (Jacobs and Azmitia, 1992). However, it is also important to note that in ketanserin-treated rats all the cholinomimetic drugs that were used in the present study decreased high-voltage spindles without actually affecting the total recording time, demonstrating that the effects of these drugs on behavioral/motor activity can be differentiated from their effects on thalamocortical oscillations and high-voltage spindles. Note also that in our previous study, a 5-HT_2 receptor agonist, DOI, decreased neocortical high-voltage spindle activity in adult rats not only after systemic injections but also when administered unilaterally directly into the ventroposteromedial thalamic area without affecting the total recording time, and this effect could be blocked by systemic administration of ketanserin (Jäkälä et al., 1995).

In conclusion, the present results showing that both the cholinergic system and 5-HT_2 receptors modulate rat neocortical high-voltage spindles are in good agreement with the results of previous in vitro studies (Pape and McCormick, 1989; McCormick, 1990, 1992; Steriade and Buzsáki, 1990; McCormick and Wang, 1991; Steriade et al., 1993), and suggest that activation of the cholinergic system and activation of 5-HT_2 receptors have at least additive effects in the suppression of rat thalamocortical oscillations, and thus in the maintenance of effective processing of information in thalamocortical systems (McCormick, 1992; Steriade et al., 1993). These data may also be of some clinical relevance, as combination drug therapies, aimed at stimulating the cholinergic and serotonergic

systems, could offer an approach for normalization of the deterioration in arousal functions related to Alzheimer's disease, as well as in a variety of other clinical disorders.

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