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Electroencephalographic activation by tacrine, deprenyl, and quipazine: cholinergic vs. non-cholinergic contributions

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Abstract

Drugs that stimulate central cholinergic transmission can induce activated, high frequency electroencephalographic (EEG) activity in rats. Monoaminergic enhancement also produces EEG activation, either by a direct stimulation of monoaminergic transmission in cortex, or a transsynaptic excitation of cholinergic projection neurons receiving excitatory monoaminergic afferents. We examined the degree of cholinergic involvement in EEG activation produced by monoaminergic and cholinergic drugs in rats. All animals were pretreated with 10 mg/kg reserpine and either 1 or 5 mg/kg scopolamine to abolish EEG activation. The acetylcholinesterase inhibitor tacrine (5–20 mg/kg) restored EEG activation in the low dose scopolamine group, but was less effective against the high scopolamine dose. The monoamine oxidase inhibitor deprenyl and the serotonergic receptor agonist quipazine restored EEG activation, an effect that was largely unaffected by different scopolamine doses. These results confirm that tacrine produces EEG activation by means of cholinergic stimulation. In contrast, activation produced by deprenyl or quipazine does not appear to be mediated by a transsynaptic excitation of cholinergic neurons and likely depends on a direct enhancement of cortical monoaminergic neurotransmission.

Keywords: EEG (electroencephalogram); Neocortex; Acetylcholinesterase inhibitor; Monoamine oxidase inhibitor; 5-HT receptor agonist; Scopolamine; Forebrain basal; (Rat)

1. Introduction

Considerable evidence suggests that the release of acetylcholine in the neocortex plays a critical role in maintaining activated, low voltage fast activity in the electroencephalogram (EEG). Early experiments measuring the release of acetylcholine in relation to the EEG showed that cortical acetylcholine levels are higher during periods of low voltage fast activity relative to synchronized large amplitude, slow activity (Kanai and Szerb, 1965; Celesia and Jasper, 1966). Electrical stimulation of the cholinergic inputs to the cortex arising in the basal forebrain (Mesulam et al., 1983; Semba and Fibiger, 1989) produces increased cortical acetylcholine release and concurrent EEG activation (Belardetti et al., 1977; Casamenti et al., 1986; Metherate et al., 1992). Conversely, basal forebrain lesions reduce EEG activation and induce a shift to large amplitude slow activity, an effect that correlates with the loss of cholinergic markers in the cortex, e.g., the enzyme choline acetyltransferase (Stewart et al., 1984; Buzsáki et al., 1988; Ray and Jackson, 1991). In addition, a large number of pharmacological studies have shown that a variety of direct and indirect cholinergic agonists can induce low voltage fast activity, while muscarinic receptor antagonists reduce both spontaneously occurring EEG activation and activation elicited by basal forebrain stimulation (Funderburk and Case, 1951; Celesia and Jasper, 1966; Cuculic et al., 1968; Metherate et al., 1992). The fact that in anesthetized preparations, stimulation-induced EEG activation is blocked by direct, intracortical application of anti-muscarinic drugs (Metherate et al., 1992) indicates that acetylcholine promotes activation by a direct, local effect on cortical neurons, rather than by some indirect, subcortical action.

Monoaminergic projections arising in the brainstem constitute a second neurochemical system thought to play an important role in maintaining EEG activation. In rats with basal forebrain lesions or given anti-muscarinic drugs, low voltage fast activity is reduced, but not abolished and continues to occur sporadically, depending on the behavioral state of the animal (Vanderwolf, 1988). This non-cholinergic, non-muscarinic activation is abolished by systemic

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treatment with the selective serotonin (5-hydroxytryptamine, 5-HT) depletor *p*-chlorophenylalanine (Vanderwolf and Baker, 1986) or intracerebral infusions of the serotonergic neurotoxin 5,7-dihydroxytryptamine (Vanderwolf et al., 1989). Importantly, intra-cortical application of several 5-HT_2 receptor antagonists (e.g., ketanserin) also blocks non-cholinergic low voltage fast activity (Neuman and Zebrowska, 1992), suggesting that 5-HT, much like acetylcholine, induces EEG activation by a direct, local action in cortex and not by some indirect, subcortical effects.

Dopamine and noradrenaline are also thought to enhance low voltage fast activity (e.g., Neuman, 1986; Ongini and Caporali, 1987; Berridge and Foote, 1991; Kropf and Kuschinsky, 1991). However, in contrast to acetylcholine and 5-HT, it appears that these transmitters are not essential for EEG activation to occur since large (up to 99%), concurrent depletions of noradrenaline and dopamine do not block activation in rats (Whishaw et al., 1978). Recently, it has been proposed that noradrenaline and dopamine modulate the EEG by an indirect, subcortical action in the basal forebrain (Dringenberg and Vanderwolf, 1998). According to this hypothesis, the release of dopamine and noradrenaline has excitatory effects on acetylcholine-containing basal forebrain neurons, resulting in an enhanced cortical acetylcholine release and, consequently, EEG activation. Several lines of evidence are consistent with this hypothesis. For example, noradrenergic and dopaminergic fibers provide a dense, possibly excitatory, innervation of the basal forebrain (Vertes, 1988; Jones and Cuello, 1989; Zaborszky, 1989). In addition, infusions of noradrenaline into the basal forebrain produce EEG activation and waking behavior (Cape and Jones, 1998), and activation induced by noradrenergic or dopaminergic stimulation (electrical or agonist-induced) is blocked by muscarinic receptor antagonists (Vanderwolf et al., 1980; Berridge et al., 1996; Dringenberg and Vanderwolf, 1997). Together, these data suggest that dopamine and noradrenaline exert an indirect control over the cortical EEG by acting on the basal forebrain and, thus, modulating the release of acetylcholine in the cortex (Dringenberg and Vanderwolf, 1998). Even the direct acting activating systems (acetylcholine and 5-HT) can exert additional, subcortical effects that allow for an indirect modulation of cortical excitability, either by actions in the basal forebrain or the thalamus (e.g., Cape and Jones, 1998; Steriade, 2000). Thus, it is likely that a complex, dynamic interplay of cortical and subcortical effects of multiple transmitter systems is involved in regulating the activation state of the mammalian cortex.

Based on the evidence summarized above, it appears that acetylcholine and 5-HT induce low voltage fast activity by a direct action in cortex, while some other transmitter substances contribute to activation by means of a transsynaptic excitation of basal forebrain cells, leading to cortical acetylcholine release. With the present experiments, we investigated the relative contributions of cholinergic and non-cholinergic mechanisms to EEG activation elicited by the monoamine oxidase inhibitor deprenyl, the 5-HT receptor agonist quipazine, and the acetylcholinesterase inhibitor tacrine as a direct, cholinergically acting reference compound. Specifically, we examined if EEG activation induced by these agents is sensitive to different doses of the muscarinic receptor antagonist scopolamine, indicative of a role of acetylcholine in mediating these activating effects.

2. Materials and methods

2.1. Subjects and surgery

All experiments were conducted in accordance with guidelines published by the Canadian Council on Animal Care and approved by the Queen's University Animal Care Committee. The experiments were conducted on adult, male Long–Evans rats (300–400 g) housed under a 12:12-h light/dark cycle with unlimited access to food and water. Rats were housed as pairs prior to surgery, and individually after surgical preparation.

Standard stereotaxic surgery was performed under sodium pentobarbital anesthesia (60 mg/kg, i.p.) and buprenorphine analgesia (0.03 mg/kg, i.p.). A chronic neocortical recording electrode (Teflon coated wire, 125 μm diameter) was implanted on the surface of the sensory-motor cortex (AP + 1.0 from bregma; L + 1.5 from midline). Reference and ground connections were placed in the bone over the cerebellum, and two to three additional anchor screws were placed in the skull to hold the head cap constructed with dental cement. All rats were given a recovery period of at least 7 days between surgery and the experiment.

2.2. EEG recordings

Neocortical EEG activity was recorded from awake, freely moving rats by means of lightweight cables attached to the implanted electrodes. Cortical activity was recorded differentially against a cerebellar connection, amplified, filtered (band pass filters at 0.3 Hz and 47 Hz), and digitized (200 Hz) by a PowerLab/4s system for the Macintosh (ADInstruments, Milford, MA). The EEG signal was displayed on one channel. Further, it was passed through additional software-controlled band-pass filters to separate and display the following frequency bands: *delta*, 0.5–4 Hz; *theta*, 4–8 Hz; and *alpha*, 8–12 Hz. For each drug treatment (including undrugged, baseline EEG activity), one artifact-free 5-s epoch (recorded during the first 5-s period of continuous immobility, as determined by close visual observation of the animal) was analyzed for the average peak-to-peak amplitude in the delta, theta, and alpha frequency bands.

2.3. Drug treatment and data collection

On the day prior to the experiment, drug-free baseline EEG recordings were taken from all rats. Approximately

14 h before further data collection, rats were given reserpine (10 mg/kg, i.p., dissolved in dimethyl sulfoxide, DMSO). Twenty-five minutes prior to data collection, rats received an injection of scopolamine hydrobromide (1 or 5 mg/kg, i.p.). Combined treatment with reserpine and an anti-muscarinic is known to produce a reliable loss of low voltage fast activity in rats (Vanderwolf et al., 1980). The doses of scopolamine used here are consistent with those employed in previous work on EEG slowing induced by muscarinic receptor blockade (Vanderwolf et al., 1980; Dringenberg et al., 2000a,b). In addition, 1 and 5 mg/kg scopolamine (in conjunction with reserpine) produce an equivalent (maximal) loss of EEG activation, ruling out consistent differences in EEG activity between different scopolamine groups prior to further drug treatments (see below). Cortical EEG activity during behavioral immobility was recorded 25 min after the scopolamine injection. Subsequently, different groups of rats were given successive, cumulative injections of one of the following drugs: tacrine (5 + 5 + 10 mg/kg, given every 25 min for a final, cumulative dose of 20 mg/kg; $n = 14$ for both the low and high scopolamine dose groups), deprenyl (5 + 5 + 10 mg/kg, every 25 min; $n = 14$ for both low and high scopolamine groups), or quipazine (5 + 5 + 10 mg/kg, every 25 min; $n = 14$ for both low and high scopolamine groups). Immobility-related EEG activity was recorded 20 min after each of these successive injections.

2.4. Data analysis

Data were analyzed by measuring average peak-to-peak amplitude in each of the three recorded frequency bands, as used previously (Dringenberg et al., 2000a,b). Analyses of filtered band frequency amplitude yield data similar to those of power spectral analyses (see Cape and Jones, 1998; Dringenberg et al., 2000a for examples). For each rat, the amplitude following scopolamine–reserpine treatment was taken as 100% and all data are expressed in relation to this maximal amplitude. Data are presented as means ± S.E.M. and were analyzed by analyses of variance and, where statistically appropriate, Newman–Keul's follow-up tests using the software package CLR ANOVA (version 1.1, Clear Lake Research).

3. Results

3.1. EEG in undrugged rats and after reserpine–scopolamine treatment

The EEG of immobile, undrugged rats consisted of low voltage fast activity of less than 0.3 mV amplitude (Fig. 1). Occasionally, bursts of synchronized, high-voltage spindle activity between 6 and 10 Hz appeared. Spindle activity is not present after cholinergic and monoaminergic blockade, and was not restored by any of the agonist drugs tested in these experiments. Thus, to ensure appropriate comparison of undrugged EEG activity and activity following reserpine–scopolamine treatment, baseline EEG epochs containing spindle activity were not included in the data analyses.

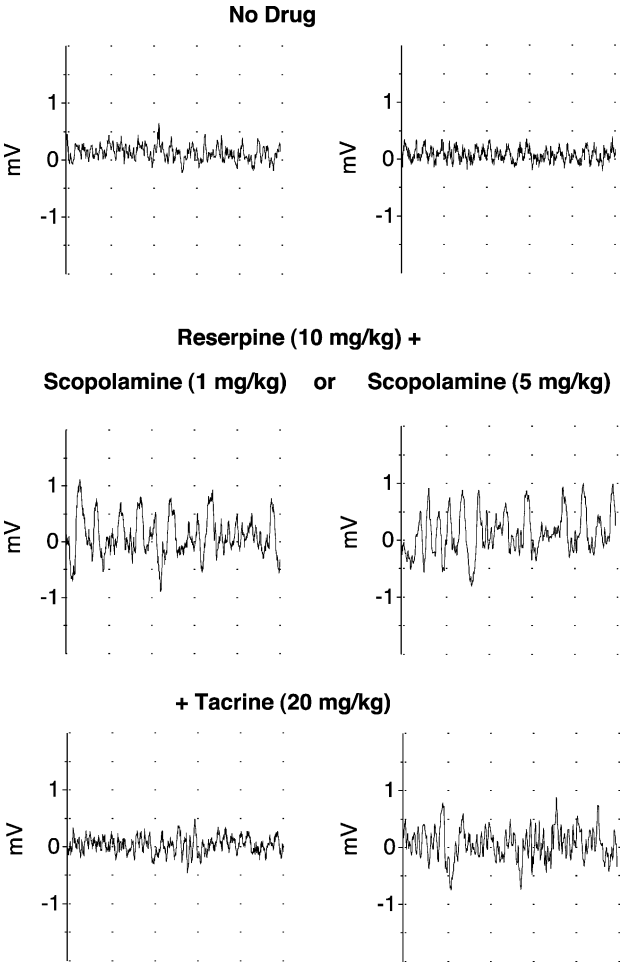

Fig. 1. Examples of raw EEG activity in untreated rats (No Drug), after combined reserpine (10 mg/kg)–scopolamine (1 or 5 mg/kg) treatment, and after additional injections of tacrine (20 mg/kg, cumulative dose). In undrugged rats, the EEG consists of low voltage fast activity. After reserpine–scopolamine treatment, the EEG is dominated by large (up to 1 mV) amplitude, slow frequency activity. Tacrine restores low voltage fast activity more effectively in rats given reserpine + 1 mg/kg scopolamine relative to rats receiving reserpine + 5 mg/kg scopolamine (EEG epochs are 5 s in duration).

The EEG of rats given reserpine (10 mg/kg) and scopolamine (1 or 5 mg/kg) shifted to large, slow activity with an amplitude of up to 1.5 mV (Fig. 1). The increase in amplitude was apparent in all three frequency bands (Fig. 2) and was not different for rats given either 1 or 5 mg/kg scopolamine (in addition to reserpine): across all experiments and relative to undrugged EEG activity, delta amplitude increased 3.2 and 2.9 fold after reserpine plus 1 or 5 mg/kg scopolamine, respectively; theta amplitude increased 3 and 2.5 fold for low and high scopolamine

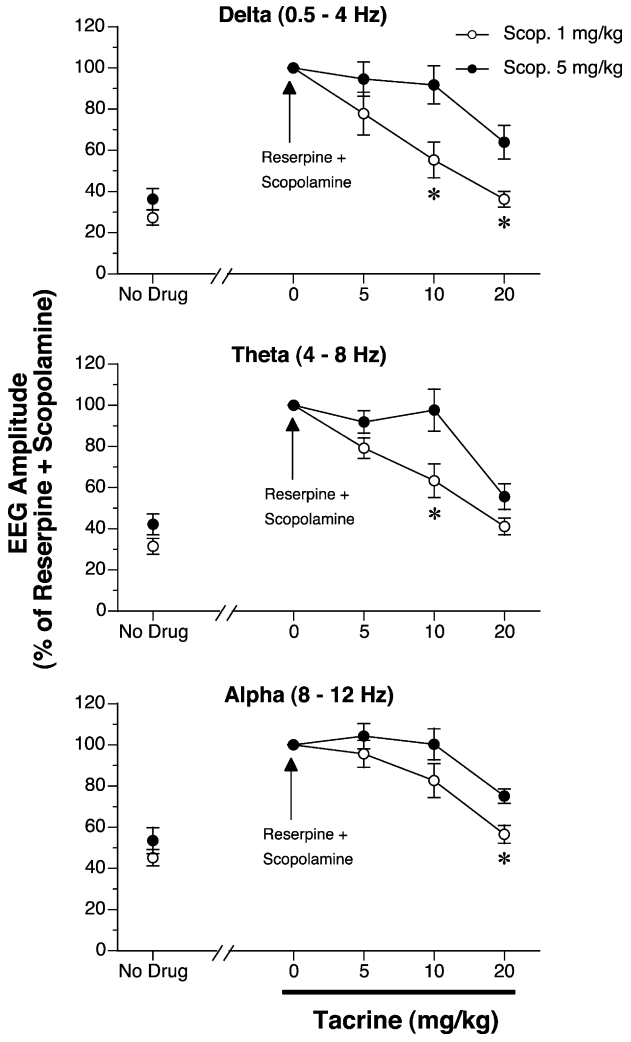

Fig. 2. EEG amplitude in the delta, theta, and alpha frequency bands and the effects of reserpine (10 mg/kg)–scopolamine (1 or 5 mg/kg, $n=14$/group) treatment, followed by successive, cumulative administration of tacrine (5+5+10 mg/kg). Reserpine–scopolamine administration increased EEG amplitude in all three frequency bands. Subsequent tacrine treatment reversed this effect more effectively in rats given scopolamine 1 mg/kg relative to scopolamine 5 mg/kg. Statistics: *Delta*: effect of scopolamine dose, $F(1,26)=7.7$, $P=0.0102$; drug (tacrine) treatment, $F(4,104)=48.8$, $P<0.0001$; scopolamine by drug treatment interaction, $F(4,104)=3.4$, $P=0.0118$. *Theta*: scopolamine dose, $F(1,26)=9.1$, $P=0.0056$; drug treatment, $F(4,104)=54.9$, $P<0.0001$, interaction, $F(4,104)=3.0$, $P=0.0211$. *Alpha*: scopolamine, $F(1,26)=4.6$, $P=0.0421$; drug, $F(4,104)=48.9$, $P<0.0001$, interaction, $F(4,104)=1.4$, $P=0.2431$ (* $P<0.05$, simple effects test).

doses, respectively; and alpha amplitude increased 2.2 and 1.9 for low and high scopolamine doses, respectively. These data suggest that reserpine plus 1 mg/kg scopolamine were sufficient to produce a maximal effect on EEG amplitude. Similar effects of reserpine–scopolamine treatment have been previously reported using both power spectral analyses and analyses methods equivalent to those used in the present experiments (Dringenberg et al., 2000a).

3.2. Effect of tacrine

Successive, cumulative administration of the acetylcholinesterase inhibitor tacrine (5+5+10 mg/kg, i.p.) suppressed large, slow EEG activity induced by reserpine–scopolamine treatment and restored cortical EEG activation (Fig. 1). However, the effect of tacrine was dependent on the dose of scopolamine used. That is, tacrine suppressed large, slow activity more effectively in rats given 1 mg/kg scopolamine relative to rats given a 5 mg/kg dose (Figs. 1 and 2). This sensitivity of the effect of tacrine to scopolamine dose was apparent for all three frequency bands examined (Fig. 2).

3.3. Effect of deprenyl

Cumulative administration of the monoamine oxidase inhibitor deprenyl (5+5+10 mg/kg, i.p.) also produced a

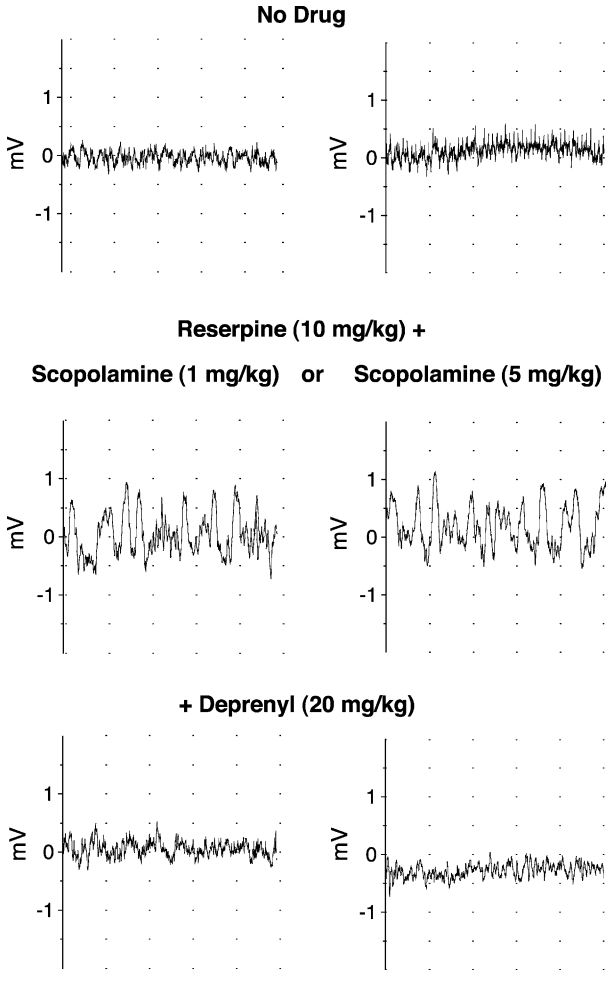

Fig. 3. EEG activity in untreated rats (No Drug), after combined reserpine (10 mg/kg)–scopolamine (1 or 5 mg/kg) treatment, and after additional injection of deprenyl (20 mg/kg, cumulative dose). Low voltage fast activity present in undrugged rats is abolished by reserpine–scopolamine treatment. Deprenyl restores low voltage fast activity equally well in rats given either 1 or 5 mg/kg scopolamine (EEG epochs are 5 s in duration).

dose-dependent reversal of large, slow activity and restoration of EEG activation in reserpine–scopolamine treated rats (Fig. 3). However, in contrast to tacrine, the effect of deprenyl was not influenced by the dose of scopolamine used (Fig. 3). Thus, for all three frequency bands, suppression of large amplitude activity by deprenyl was not different for rats given either 1 or 5 mg/kg of scopolamine (Fig. 4).

3.4. Effect of quipazine

Cumulative administration of the 5-HT receptor agonist quipazine (5 + 5 + 10 mg/kg, i.p.) also reversed large, slow activity and restored EEG activation in rats given reserpine–scopolamine. Similar to the effect of deprenyl, the effect of quipazine was not affected by the dose of scopolamine used (Fig. 5). That is, for all three frequency bands, the suppression of large amplitude activity by quipazine was not different for rats given either 1 or 5 mg/kg scopolamine (Fig. 5).

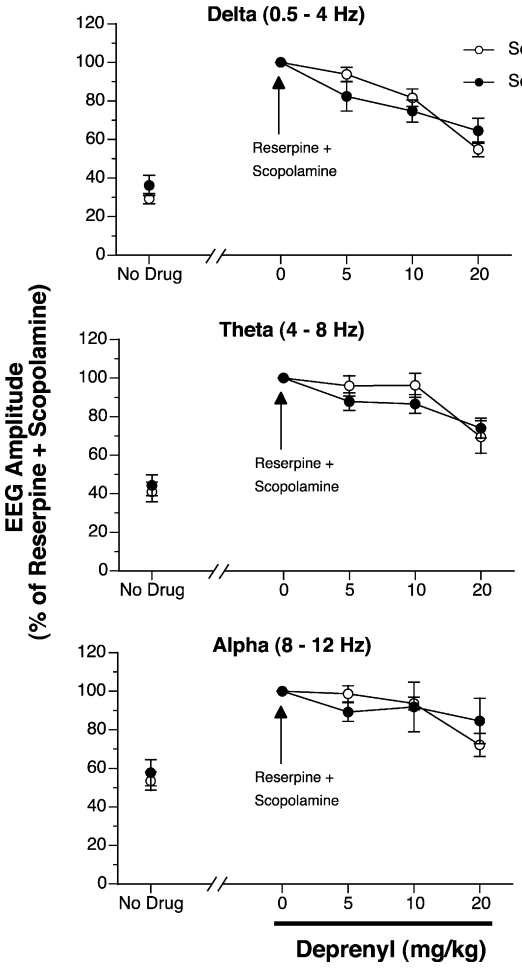

Fig. 4. EEG amplitude in the delta, theta, and alpha frequency bands and the effects of reserpine (10 mg/kg)–scopolamine (1 or 5 mg/kg, $n = 14$/group) treatment, followed by successive, cumulative administration of deprenyl (5 + 5 + 10 mg/kg). Reserpine–scopolamine administration increased EEG amplitude in all three frequency bands. Subsequent deprenyl treatment reversed the effect of reserpine–scopolamine, and this effect was not different for rats receiving 1 or 5 mg/kg scopolamine. Statistics: *Delta*: scopolamine dose, $F(1,26) = 0.006$, $P = 0.9399$; drug (deprenyl) treatment, $F(4,104) = 100.4$, $P < 0.0001$; scopolamine by drug interaction, $F(4,104) = 2.9$, $P = 0.0265$. *Theta*: scopolamine dose, $F(1,26) = 0.2$, $P = 0.691$; drug treatment, $F(4,104) = 61.3$, $P < 0.0001$, interaction, $F(4,104) = 1.2$, $P = 0.2974$. *Alpha*: scopolamine, $F(1,26) = 0.03$, $P = 0.8737$; drug, $F(4,104) = 22.4$, $P < 0.0001$, interaction, $F(4,104) = 1.2$, $P = 0.3386$.

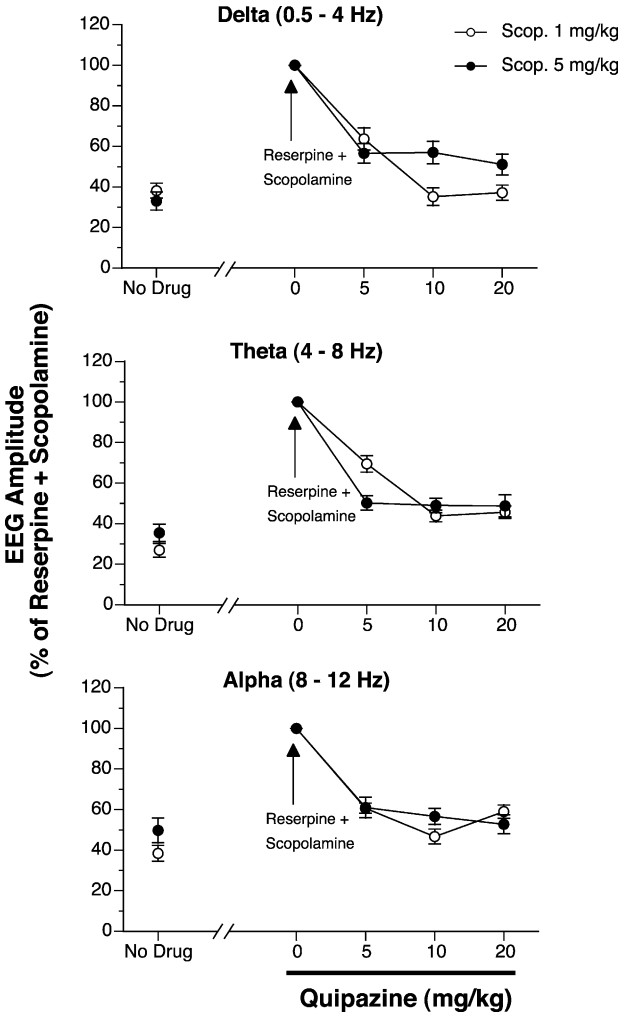

Fig. 5. EEG amplitude in the delta, theta, and alpha frequency bands and the effects of reserpine (10 mg/kg)–scopolamine (1 or 5 mg/kg, $n = 14$/group) treatment, followed by successive, cumulative administration of quipazine (5 + 5 + 10 mg/kg). Reserpine–scopolamine administration increased EEG amplitude in all three frequency bands. Subsequent quipazine treatment reversed the effect of reserpine–scopolamine, and this effect was not different for rats receiving 1 or 5 mg/kg scopolamine. Statistics: *Delta*: scopolamine dose, $F(1,26) = 2.6$, $P = 0.1203$; drug (quipazine) treatment, $F(4,104) = 139.4$, $P < 0.0001$; scopolamine by drug interaction, $F(4,104) = 1.3$, $P = 0.2765$. *Theta*: scopolamine, $F(1,26) = 2.7$, $P = 0.1157$; drug, $F(4,104) = 176.4$, $P < 0.0001$, interaction, $F(4,104) = 1.4$, $P = 0.2287$. *Alpha*: scopolamine, $F(1,26) = 0.475$, $P = 0.4968$; drug, $F(4,104) = 72.7$, $P < 0.0001$, interaction, $F(4,104) = 0.3$, $P = 0.9101$.

4. Discussion

A large variety of cholinergic and monoaminergic agonists are effective in suppressing large amplitude, synchron-

ized EEG activity, replacing it with activated low voltage fast activity. The present experiments confirm that the acetylcholinesterase inhibitor tacrine, the monoamine oxidase inhibitor deprenyl, and the 5-HT receptor agonists quipazine all suppress large, slow activity induced by combined reserpine–scopolamine treatment in the rat (Dringenberg and Vanderwolf, 1996; Dringenberg et al., 2000a,b). These observations suggest that both cholinergic and monoaminergic systems play important roles in regulating the activation state of the mammalian cortex.

Large amplitude, slow oscillatory activity can be observed in isolated, large slabs of neocortical tissue, suggesting that intrinsic cortical networks can sustain this type of slow, deactivated cortical activity (Timofeev et al., 2000). In fact, recent data suggest a presence of two distinct types of large amplitude, low frequency rhythms in the EEG of cats and humans, one in the typical delta range (1–3 or 4 Hz), and a second, slow oscillations below 1 Hz (Steriade et al., 1993a,b; Achermann and Borbely, 1997). Activated low voltage fast activity is not present in the isolated cortex, indicating that subcortical inputs are necessary for the induction of EEG activation. Cholinergic fibers from the basal forebrain that terminate in the cortex play a major role in reducing slow oscillatory cortical activity. During slow, synchronized activity, cortical pyramidal cells display low frequency intracellular membrane oscillations and pronounced, long lasting inhibitory after-hyperpolarizations following spike discharge (Buzsáki and Gage, 1989; Metherate et al., 1992). Extracellular currents associated with these slow, synchronized events are believed to summate in the extracellular fluid, resulting in the appearance of large amplitude, low frequency EEG activity. Acetylcholine, by acting on intracortical muscarinic receptors, but not nicotinic binding sites, blocks slow intracellular membrane oscillations and the outward potassium current associated with inhibitory after-hyperpolarizations; thus blocking slow, synchronized activity and facilitating a shift to cortical activation (Buzsáki and Gage, 1989; Metherate et al., 1992; Steriade et al., 1993a,b).

The basal forebrain cholinergic system itself is under powerful, modulatory control by fibers arising in the brainstem and diencephalon, including noradrenergic, dopaminergic, serotonergic, histaminergic, and brainstem cholinergic inputs (Vertes, 1988; Jones and Cuello, 1989; Semba and Fibiger, 1989; Zaborszky, 1989). The available evidence suggests that these transmitters can indirectly modulate cortical EEG activity by an action in the basal forebrain that affects basal forebrain–cortical impulse flow. For example, direct infusions of noradrenaline into the basal forebrain facilitate high frequency EEG activation and waking behavior in rats (Cape and Jones, 1998). Cortical low voltage fast activity elicited by electrical stimulation of the locus coeruleus is blocked by systemic anti-muscarinic treatment, suggesting a critical role of acetylcholine in mediating this activating effect (Dringenberg and Vanderwolf, 1997). Similarly, basal forebrain infusions of histamine or histamine H1 receptor agonists result in increases in cortical acetylcholine release (Cecchi et al., 2001). Thus, it is clear that at least some of the effects of noradrenaline and histamine on cortical activity are indirect, mediated by the basal forebrain cholinergic system. The fact that depletions of histamine, dopamine, or noradrenaline do not result in a loss of low voltage fast activity (Whishaw et al., 1978; Servos et al., 1994) suggests that these systems have a modulatory role, but are not essential for the maintenance of activation (see Dringenberg and Vanderwolf, 1998).

As mentioned above, systemic treatment with drugs that enhance monoaminergic transmission such as the monoamine oxidase inhibitors pargyline, tranylcypromine, or deprenyl is effective in inducing cortical low voltage fast activity (Vanderwolf et al., 1980; Vanderwolf, 1988; Dringenberg et al., 2000a,b). Deprenyl, at the concentrations used in the present investigation, inhibits both monoamine oxidase-A and monoamine oxidase-B, resulting in nonspecific increases in brain monoamine levels (Volz and Gleiter, 1998; ThyagaRajan and Quadri, 1999; also see Schneider et al., 1991). Consequently, the EEG effects of deprenyl may be due to a stimulation of serotonergic, dopaminergic, and/or noradrenergic transmission.

As mentioned previously, EEG activation induced by selective noradrenergic or dopaminergic stimulation (electrical or agonist-induced) is blocked by muscarinic receptor antagonists (Vanderwolf et al., 1980; Berridge et al., 1996; Dringenberg and Vanderwolf, 1997), suggesting that the action of these transmitters to induce activation is mediated by acetylcholine. In fact, direct infusions of noradrenaline or adrenergic agonists into the basal forebrain area elicit EEG activation in anesthetized and freely moving rats (Berridge et al., 1996; Cape and Jones, 1998). In contrast, 5-HT-dependent EEG activation is insensitive to large doses of muscarinic receptor antagonists (e.g., 5 and 50 mg/kg scopolamine and atropine, respectively; Vanderwolf et al., 1980, 1989). In the present experiment, deprenyl restored low voltage fast activity in rats pretreated with reserpine and scopolamine, and this effect was independent of the dose of scopolamine used (1 or 5 mg/kg). This insensitivity of deprenyl-induced activation to scopolamine suggests that it is not mediated by a cascade of dopamine and/or noradrenaline stimulation, eliciting an increase in cortical acetylcholine release. Rather, activation of the EEG after deprenyl treatment appears to depend on serotonergic enhancement, known to be insensitive to cholinergic blockade. In this respect, it is important to note that the acetylcholinesterase inhibitor tacrine restored low voltage fast activity more effectively in rats given 1 mg/kg scopolamine relative to rats given a 5 mg/kg dose. These data confirm the sensitivity of acetylcholine-dependent activation to the scopolamine doses used in the present study.

Consistent with previous work (Dringenberg and Vanderwolf, 1996), the serotonergic receptor agonist quipazine also restored EEG activation after reserpine–scopolamine treatment. Further, much like the effect observed with

deprenyl, activation induced by quipazine was not affected by the different scopolamine doses used. As discussed, there is clear evidence that 5-HT can produce low voltage fast activity independent of the cholinergic system. In fact, EEG activation that is maintained after cholinergic blockade critically depends on 5-HT since it is abolished by the 5-HT depletor *p*-chlorophenylalanine or lesions with the 5-HT neurotoxin 5,7-dihydroxytryptamine (Vanderwolf and Baker, 1986; Vanderwolf et al., 1989). The fact that 5-HT produces low voltage fast activity by a direct, intracortical action is suggested by experiments showing that activation is abolished by 5-HT_2 receptor antagonists applied locally in the cortex (Neuman and Zebrowska, 1992). Quipazine acts as a relatively non-selective ligand with activity at both presynaptic (autoreceptor) and postsynaptic 5-HT binding sites, including 5-HT_1, 5-HT_2, and 5-HT_3 receptor types (Blier and de Montigny, 1983; Peroutka, 1990). However, given the role of cortical 5-HT_2 receptors identified in previous work, it is likely that this receptor type plays a role in mediating the effects of quipazine observed here.

The present experiments demonstrate that doses of 1 and 5 mg/kg scopolamine produced equivalent slowing of the EEG. Thus, the high scopolamine dose used, which failed to differentially affect restoration by deprenyl and quipazine, was at least five-fold higher than the dose required to produce a maximal effect on cholinergic-dependent EEG activation. Based on this evidence, it seems unlikely that even higher scopolamine doses would reveal a cholinergic (scopolamine-sensitive) component of EEG activation elicited by deprenyl or quipazine. However, examination of the dose–response curves suggests that quipazine appeared to be more effective than tacrine in restoring EEG activation. This leftward shift in the dose–response curve for quipazine raises the possibility that lower quipazine doses may be more sensitive to an increase in scopolamine dose. Even though it is difficult to rule out this possibility, it is worthwhile mentioning that quipazine and tacrine at doses of 5 and 10 mg/kg, and of 10 and 20 mg/kg, respectively, produced roughly equivalent effects on the EEG. For these doses, we found clear evidence for a differential effect of scopolamine against tacrine, but not quipazine. Thus, the differential effect of scopolamine is maintained when doses of these two drugs are matched for effectiveness. Future experiments should be able to resolve this issue by including lower doses of quipazine than those used in the present study.

A loss of high frequency EEG activation and the appearance of slow waves are typical physiological markers associated with senile dementia and Alzheimer's disease (Penttilä et al., 1985; Rodriguez et al., 1999). Further, these EEG changes correlate well with the appearance and progression of cognitive decline in dementia patients (Penttilä et al., 1985; Rodriguez et al., 1999). Centrally acting acetylcholinesterase inhibitors, including tacrine, are commonly used in the treatment of Alzheimer's disease. Acute or chronic tacrine administration can reduce excessive, low frequency EEG activity in the delta and theta bands in Alzheimer's disease patients (Jelic et al., 1998; Knott et al., 2000). However, the clinical effectiveness of acetylcholinesterase inhibition is variable and temporally limited (see Francis et al., 1999 for review). In part, the continuing, progressive loss of cholinergic inputs to the cortex may account for the limited success with acetylcholinesterase inhibition. The present results demonstrate that monoaminergic and serotonergic drugs can produce a restoration of cortical activation, and that this effect is independent of the cholinergic system. If these data are confirmed, monoaminergic agonists may provide a novel pharmacological approach to restore some aspects of cortical functioning that does not depend on the level of remaining cholinergic innervation of the cortex. However, thorough testing of this hypothesis in future studies is required to assess its validity.

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