Serotonin 5-HT$_{2A}$ receptors have been implicated in psychiatric illness and the psychotomimetic effects of hallucinogens. In brain slices, focal stimulation of 5-HT$_{2A}$ receptors in rat prefrontal cortex results in dramatically increased glutamate release onto layer V pyramidal neurons, as measured by an increase in “spontaneous” (nonelectrically evoked) EPSCs. This glutamate release is blocked by tetrodotoxin (TTX) and is thought to involve local spiking in thalamocortical axon terminals; however, the detailed mechanism has remained unclear.

Here, we investigate parallels in EPSCs induced by either serotonin or the potassium channel blockers 4-aminopyridine (4-AP) or ß-dendrotoxin (DTX). DTX, a selective blocker of Kv1.2-, and Kv1.6-containing potassium channels, has been shown to release glutamate in cortical synaptosomes, presumably by inhibiting a subthreshold-activated, slowly inactivating potassium conductance. By comparing DTX with other potassium channel blockers, we found that the ability to induce EPSCs in cortical pyramidal neurons depends on affinity for Kv1.2 subunits. DTX-induced EPSCs are similar to 5-HT-induced EPSCs in terms of sensitivity to TTX and ß-agatoxin-IVA (a blocker of P-type calcium channels) and laminar selectivity. The involvement of thalamocortical terminals in DTX-induced EPSCs was confirmed by suppression of these EPSCs by µ-opioid and thalamic lesions. More directly, DTX-induced EPSCs substantially occlude those induced by 5-HT, suggesting a common mechanism of action. No occlusion by DTX was seen when EPSCs were induced by a nicotinic mechanism. These results indicate that blockade of Kv1.2-containing potassium channels is part of the mechanism underlying 5-HT-induced glutamate release from thalamocortical terminals.

Key words: K$^+$; voltage-gated; dendrotoxin; 5-hydroxytryptamine; 5-HT$_{2A}$ receptor; psychedelic hallucinogens; 4-aminopyridine; prefrontal
spiking in cortical synaptosomes (Tibs et al., 1989, 1996) and certain populations of axon terminals but has little direct effect on currents in the soma of the presynaptic cell (Southan and Robertson, 1998, 2000; Bekkers and Delaney, 2001).

Here, we show that DTX mimics the effects of 5-HT by inducing EPSCs preferentially in layer V pyramidal neurons. A comparison of effects of DTX and other potassium channel blockers reveals selectivity of EPSC induction by blockers with affinity for Kv1.2 subunits. DTX-induced EPSCs meet many general tests of similarity to 5-HT-induced EPSCs. Most interestingly, however, DTX substantially occludes EPSCs induced by 5-HT, suggesting a common mechanism of action.

MATERIALS AND METHODS

Preparation of prefrontal cortical slice. Brain slices were prepared from 3- to 5-week-old male Sprague Dawley albino rats, in adherence with protocols approved by the Yale University Animal Care and Use Committee. All efforts were made to minimize both the number of animals used and their suffering.

Briefly, rats were deeply anesthetized with chloral hydrate (400 mg/kg) and decapitated. Brains were quickly removed and blocked in ice-cold oxygenated modified artificial CSF (ASC) in which sucrose (252 mM) is substituted for NaCl. A 300-μm-thick hemispherical section was cut on a DSK microslicer (Dosaka EM, Kyoto, Japan) and transferred to the stage of a submerged recording chamber perfused with fast-flowing (4 ml/min) oxygenated, standard ACSF; the slice was secured by a fine mesh attached to a platinum wire frame. Standard ACSF was composed of (in mM): 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 10 d-glucose, 25 NaHCO₃, 2 CaCl₂, and 2 MgSO₄, pH 7.35.

Whole-cell recordings of EPSCs in prefrontal pyramidal neurons. Medial prefrontal pyramidal cells were selected using an Olympus BX50WI (40× infrared lens; numerical aperture, 0.8) with infrared differential interference contrast microscopy (Olympus, Melville, NY), as described by Stuart et al. (1993). Low-resistance patch pipettes (3–5 MΩ) were pulled from Kvar glass tubing (World Precision Instruments, Sarasota, FL) using a Brown and Flaming horizontal puller (Sutter Instruments, Novato, CA) and filled with the following pipette solution (in mM): 120 K-glucinate, 10 HEPEs, 5 BAPTA K₄, 20 sucrose, 2.38 CaCl₂, 1 MgCl₂, 1 Na₂ATP, and 0.1 Tris-GTP, pH 7.33. Somatic recordings were made in current-clamp (bridge) mode with an Axonclamp-2B amplifier (Axon Instruments, Foster City, CA) and yielded mean resting potential, spike amplitude, and input resistance values of −72.3 ± 3.1 mV, 104.9 ± 10.3 mV, and 55.7 ± 20.2 MΩ, respectively.

Synaptic currents were recorded using continuous single-electrode

Table 1. EPSCs induced by serotonin (20 μM, 40 sec), DTX (200 nM, 10 min), and 4-AP (100 μM, 4 min) have similar kinetics (five cells per condition, 30 single peaks per cell)

<table>
<thead>
<tr>
<th>Condition</th>
<th>10–90 Rise (msec)</th>
<th>σ (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>1.0 ± 0.1</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>DTX</td>
<td>1.1 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>4-AP</td>
<td>1.1 ± 0.1</td>
<td>3.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 2. EPSCs induced by serotonin and DTX are suppressed to similar degrees by blockade of (1) TTX-sensitive sodium channels, (2) ω-agatoxin-sensitive, high-voltage-activated calcium channels, and (3) LY293558-sensitive AMPA/kainate receptors

<table>
<thead>
<tr>
<th>Condition</th>
<th>Somatic Conductance (nA/nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>−98 ± 2%</td>
</tr>
<tr>
<td>ω-Agatoxin IVA</td>
<td>−95 ± 1%</td>
</tr>
<tr>
<td>LY293558</td>
<td>−99 ± 1%</td>
</tr>
<tr>
<td>4-AP</td>
<td>−72 ± 2%</td>
</tr>
<tr>
<td>ω-Agatoxin IVA</td>
<td>−95 ± 3%</td>
</tr>
<tr>
<td>LY293558</td>
<td>−99 ± 1%</td>
</tr>
</tbody>
</table>

TTX, 2 μM, 5 min (n = 4); ω-agatoxin IVA, 200 nM, 10 min (n = 4); and LY293558, 3 μM, 5 min (n = 4).
voltage-clamp mode. Neurons were held at approximately -75 mV, and access resistance of ≈8 MΩ was maintained throughout the recording for all cells included in this study. Spontaneously occurring EPSCs were low-pass filtered at 3 kHz, amplified through cyberamp, digitized at 15 kHz, and acquired using pClamp/Digidata 1200 (Axon Instruments).

Data analysis. Analysis of EPSCs from each 10 sec block of sweeps was performed using MiniAnalysis software (Synaptosoft Inc., Decatur, GA). This program detects and measures spontaneous synaptic events according to amplitude, rise time, decay time, and area under the curve. Because of high frequency of EPSCs, the ability to accurately measure overlapping or closely occurring peaks is important for our analysis. The software uses an algorithm to detect multiple and complex peaks and automatically adjusts the baseline of closely occurring peaks using exponential extrapolation of decay. Amplitude and area thresholds were set to 8 pA and 25–50 fC, respectively. For drug conditions, EPSCs were recorded continuously so that the 10 sec peak period of EPSCs (for 5-HT) or a stable plateau (for DTX) could be detected. 5-HT-induced EPSCs had a rapid onset, reaching a peak at 40 sec, whereas DTX required 8–10 min before reaching a plateau. To avoid desensitization, 5-HT was applied for 1 min each time. Once the peak period was noted for a specific condition, the same measurement period was used for all cells included in a specific experiment. For analysis of EPSC kinetics, we sampled 30 isolated single peaks of varying amplitudes from each condition, e.g., basal, 5-HT, or DTX. Time constants of decay were estimated by single-exponential curve fitting.

Radio-frequency lesions. We followed the radio-frequency lesioning procedure described by Marek et al. (2001). In brief, rats were deeply anesthetized with an intraperitoneal injection of 400 mg/kg chloral hydrate and placed in a stereotaxic apparatus. An insulated stainless-steel electrode (with 2 mm of tip exposed) was lowered through a small burr hole into the anterior thalamus (coordinates from bregma: 2.5 mm posterior, 1.2 mm lateral, and 6.0 mm depth). Unilateral lesions were made by passing 20 mA of current for 60 sec using a Grass Instruments (West Warwick, RI) LM4 radio-frequency lesion maker (100 kHz). Sham-operated rats received the same treatment; the electrode was lowered 1 mm short of the desired target and current was not passed. Animals were allowed a 10–14 d recovery period to allow the degeneration of thalamocortical projections. The lesions were reconstructed along mediolateral, dorsoventral, and anteroposterior dimensions using coronal sections from Paxinos and Watson (1986).

Chemicals and toxins. The following toxins were from Alomone Labs (Jerusalem, Israel): TTX, DTX, r-agitoxin-2, toxin K, r-margatoxin, and r-iberiotoxin. All other compounds, including tetraethylammonium (TEA), [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO), and methionine enkephalin, were from Sigma (St. Louis, MO). LY293558 was a gift from Eli Lilly & Co. (Indianapolis, IN).

RESULTS

Low concentrations of the voltage-gated potassium channel blockers DTX and 4-aminopyridine (4-AP) were found to induce EPSCs that closely resemble those induced by 5-HT in the same cells, as shown in Figure 1. This resemblance was further char-
characterized through kinetic analysis of 10–90% rise time and 63% decay time (t), illustrated in Table 1. Both 5-HT- and DTX-induced EPSCs can be suppressed by fast sodium channel block with TTX (2 μM), P-type, high-voltage-activated calcium channel block with ω-agatoxin IVA (200 nM), and AMPA receptor block with LY293558 (3 μM), as shown in Table 2 (application times and number of cells are given in the table legend). After these basic similarities had been established, additional parallels between effects of 5-HT and stimulation and voltage-gated potassium channel block were explored in experiments described below.

In contrast to their consistent effects on EPSCs, postsynaptic effects of DTX and 5-HT varied. Typically, 5-HT induced ~5 mV depolarization, although small hyperpolarizations occurred in a small number of cells. After prolonged exposure, DTX induced a small postsynaptic depolarization of ~3 mV in some cells, but others showed no change in resting potential. These postsynaptic changes in membrane potential induced by either 5-HT or DTX were dissociated in time from induction of EPSCs, in most cases not beginning until 30 sec after the onset of 5-HT-induced EPSCs and several minutes after the onset of DTX-induced EPSCs. No cell was depolarized to spike threshold by either 5-HT or DTX.

**Kv channel subfamily and subunit specificity**

We used several different general and specific potassium channel blockers to establish the selectivity and specificity of the ability of these agents to induce EPSCs. As shown in Figure 2A, only DTX and the broader-spectrum voltage-gated potassium channel blocker 4-AP (100 μM; application times and number of cells are given in the figure legends) were able to induce EPSCs. TEA, a more general blocker of potassium channels, was unable to induce a significant increase in EPSCs at 0.5–3 mM. Toxins with high affinity for certain subunits of the Kv1 subfamily were tested to ascertain which were critical for the induction of EPSCs, as illustrated in Table 3. DTX, a high-affinity blocker of Kv1 channels containing Kv1.1, Kv1.2, or Kv1.6 subunits (Harvey, 1997) induced EPSCs at a level comparable with 5-HT. In contrast, r-agatoxin-2 (30 nm), a high-affinity blocker of Kv1 channels containing Kv1.1, Kv1.3, or Kv1.6 (Garcia et al., 1994), but not Kv1.2, failed to induce EPSCs. This difference suggests that the Kv1.2 subunit may be essential for the DTX-induced EPSCs we observe. Toxin K (200 nm) and r-margatoxin (30 nm), blockers of Kv1.1 and Kv1.3, respectively, failed to produce significant increases in EPSCs. Barium chloride (100 μM), a blocker of inwardly rectifying potassium channels, also failed to produce significant increases in EPSCs, despite its ability to produce intermittent paroxysmal depolarizing shifts. Such shifts were not seen with 5-HT or DTX.

In another group of cells (n = 9), within-cell comparisons show that EPSCs induced by a stable, maximal level of DTX (200 nm, 10 min) were significantly correlated (r = 0.9; R² = 0.5; df = 8; p < 0.01) with those induced by 5-HT (20 μM, 40 sec).

**Laminar differences in EPSC induction**

5-HT preferentially induces EPSC in layer V neurons compared with levels induced in neurons in layers II/III or V1 (Lambe et al., 2000). EPSCs induced by voltage-gated potassium channel blockade and by 5-HT were similar in neurons in each layer, as demonstrated in Figure 2B. For these experiments, 4-AP (100 μM) rather than DTX was used because the former can be washed out, allowing testing of cells in multiple layers in the same slice. Both 4-AP and 5-HT preferentially induced EPSCs in layer V. In a small sample of neurons, EPSCs induced by 4-AP and DTX were compared in the different laminae: each produced a similar level of EPSCs (data not shown). This laminar specificity of EPSCs induced by DTX and low concentrations of 4-AP suggests that only a small subset of axon terminals in the cortex are affected.

**Suppression by μ-opioid agonists**

μ-Opiates are thought to selectively inhibit glutamate release from thalamocortical terminals (Sahin et al., 1992; Delfs et al., 1994; Mansour et al., 1994; Vogt et al., 1995; Marek et al., 2001). Previously, it has been shown that μ-opioid agonists markedly suppress 5-HT-induced EPSCs (Marek and Aghajanian, 1998). In the present study, the μ-opiate receptor agonist DAMGO (1 μM, 4 min; n = 4) was shown to suppress DTX-induced EPSCs, as illustrated in Figure 3A. The nonselective, but quickly metabolized, opiate receptor agonist enkephalin (100 μM) suppressed to a similar degree EPSCs induced by 5-HT, 4-AP, or DTX, as shown in Figure 3B.

**DTX-induced EPSCs are greatly reduced by thalamic lesions**

Previously, it has been shown that thalamic lesions markedly reduce 5-HT-induced EPSCs (Marek et al., 2001). We made unilateral radio-frequency lesions in the anterior thalamus in four animals, as illustrated in Figure 4, and performed sham operations in two animals. In slices from lesioned animals, one of nine induced responses were not expected to be completely eliminated. The reduction in 5-HT-induced EPSCs is similar to that seen previously (Marek et al., 2001). The latter study showed that control lesions of the amygdala did not reduce 5-HT-induced EPSCs (Marek et al., 2001).

**Occlusion of 5-HT-induced EPSCs by potassium channel blockade**

To explore more directly the question of whether 5-HT and DTX induce EPSCs through a common mechanism, we used DTX to induce a stable level of EPSCs and then probed with a test pulse of 5-HT to see whether this combination was additive. If 5-HT induces EPSCs through an inhibition of Kv1.2, then DTX should be able to occlude the effects of 5-HT. Figure 5 reveals that the effects of 5-HT were substantially (65%) occluded by DTX (200 nm). A paired t test revealed a significant difference between EPSCs induced by the combination of 5-HT and DTX and the

| Table 3. Only compounds able to block Kv1.2 (i.e., 4-AP and DTX) induce EPSCs similar to those induced by 5-HT |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 4-AP        | X               | X               | X               | X               | X               |                 |                 |
| DTX         | X               | X               |                 |                 |                 |                 |                 |
| Agitoxin    | X               | X               |                 |                 |                 |                 |                 |
| Toxin K     | X               |                 |                 |                 |                 |                 |                 |
| Margatoxin  |                 | X               |                 |                 |                 |                 |                 |
| TEA         | X               |                 | X               |                 |                 |                 |                 |
| Ba²⁺        |                 |                 |                 |                 | X               |                 |                 |

From Coetzee et al. (1999) and Harvey (1997).
hypothesized additive value (paired t test; t = 6.9; df = 8; p < 0.001).

Although the lower-than-additive level of EPSCs with the combination of DTX and 5-HT looks like occlusion, there may be other reasons why the total is not additive, such as a physiological or measurement ceiling effect. To determine specificity of the occlusion of 5-HT-induced EPSCs by DTX, we took advantage of the nicotinic acetylcholine receptor as an alternative means to depolarize the thalamocortical terminals involved in the 5-HT effect (Lambe and Aghajanian, 2001). Like μ-opioid binding, nicotinic binding in midcortical layers is substantially reduced after thalamic lesions and is not changed by cortical excitotoxic lesions (Sahin et al., 1992; Lavine et al., 1997), indicating that high-affinity nicotinic acetylcholine receptors in those layers are present primarily on thalamocortical terminals. Stimulation of ionotropic nicotinic acetylcholine receptors with nicotine or ace-

Figure 3. DTX-induced EPSCs are suppressed by μ-opioid agonists. A, Recordings from one cell during baseline (1), DTX (2) (200 nM, 10 min), and DAMGO (3) (1 μM; 4 min). B, Comparison of the ability of enkephalin (100 μM, 3 min) to suppress EPSCs induced by 5-HT, 4-AP, or DTX (n = 5 cells per condition).

Figure 4. Unilateral radio-frequency lesions were made in the anterior thalamus, as illustrated on the left. After 10–14 d recovery to allow degeneration of thalamocortical terminals, ex vivo studies were performed in prefrontal cortex slice, and responses to 5-HT and DTX were assessed. Neurons from animals with thalamic lesions show greatly reduced EPSCs induced by either 5-HT (20 μM, 40 sec; n = 15) or DTX (200 nM, 10 min; n = 9) compared with those from sham-operated or naïve animals. There were no significant differences between neurons from sham-operated (n = 5) and surgery-naïve (n = 10) animals.

Figure 5. If 5-HT (20 μM, 40 sec) and DTX (200 nM, 10 min) induce EPSCs through different mechanisms, together their effects should be additive. Instead, the combination of 5-HT and DTX (40 sec application once plateau of DTX-induced EPSCs has been reached) is ~65% occluded compared with the theoretical additive value (n = 9).

Figure 5. Possible involvement of a high-voltage- or calcium-activated potassium channel

As noted above, occlusion of the 5-HT effect by DTX was substantial but not complete. Within-cell comparisons before and during the test pulse of 5-HT in the presence of DTX (n = 9) showed that 5-HT induced a small but consistent increase in EPSC frequency and amplitude (paired t test; t = 8.3; df = 8; p < 0.001). We observed that a low concentration of TEA (500 μM, 1 min), which had no effect on its own, increased the level of EPSCs from the DTX baseline to a level similar to a test pulse of 5-HT (r = 0.9; R^2 = 0.8; df = 8; p < 0.002) and almost completely
Additional experiments are needed to discern which of the channels susceptible to block by micromolar levels of TEA may be involved. These include the large-conductance calcium-activated potassium current BK and members of the high-voltage-activated Kv3 subfamily (Coetzee et al., 1999). BK is expressed in many parts of brain (Chang et al., 1997; Wanner et al., 1999; Behrens et al., 2000), including prominent expression in thalamus (Chang et al., 1997). BK appears to be targeted to axons and nerve terminals (Knaus et al., 1996) in which it is likely involved in modulating presynaptic calcium signals and transmitter release (Robitaille and Charlton, 1992). In this study, we found that iberiotoxin (200 nM, 15 min; n = 4), a selective inhibitor of BK, failed to increase EPSCs above the level of DTX alone (data not shown). However, recent studies (Behrens et al., 2000; Meera et al., 2000; Weiger et al., 2000) suggest that the bulky neuronal β4 subunits render BK insensitive to iberiotoxin. As a result, the latter agent does not provide a definitive test of the potential involvement of BK in 5-HT-induced EPSCs.

DISCUSSION

Here we describe striking similarities in kinetics, distribution, and pharmacology between 5-HT-induced EPSCs and those induced by DTX and 4-AP. These effects are specifically associated with Kv1.2 blockade and are not induced by available blockers of other Kv1 potassium channel subunits. The ability of μ-opioids and thalamic lesions to substantially reduce DTX-induced EPSCs confirms that DTX is acting on thalamocortical terminals. The selective occlusion of 5-HT-induced EPSCs by DTX suggests that blockade of Kv1.2-containing potassium channels is part of the mechanism underlying 5-HT-induced glutamate release from thalamocortical terminals.

Anatomical localization of DTX binding and Kv1.2

We found that only the potassium channel blockers we tested with high affinity for Kv1.2 subunits were able to induce appreciable EPSCs, as shown in Figure 2. Wang et al. (1994) and Sheng et al. (1994) have shown that Kv1.2 antibody staining in prefrontal
The issue of channel subunit composition in specific cell types or the issue of preferential targeting within a certain type of neuron.

Because most toxins have been only tested for affinity to homomeric channels in expression systems, the inability of a certain toxin (i.e., toxin K) to induce EPSCs does not rule out the involvement of a particular subunit in a heteromeric channel (i.e., Kv1.1). Strikingly, Kv1.1, which has been found together with Kv1.2 subunits in axons and terminals (Monaghan et al., 2001), can be inhibited in expression systems by G_q-coupled 5-HT receptors (Imbrici et al., 2000).

**Occlusion**

The ability of DTX to occlude 5-HT induced EPSCs is consistent with 5-HT acting to block Kv1.2. However, without a positive control, a physiological or measurement ceiling effect cannot be ruled out. To ascertain the selectivity of the occlusion of 5-HT by DTX, we used nicotinic receptor stimulation as an alternative means to depolarize thalamocortical terminals affected by 5-HT and DTX (Lambe and Aghajanian, 2001). Nicotinic receptors are ionotropic and conduct a mixed cation current. The combination of DTX and acetylcholine was additive, as would be expected from depolarization through two independent mechanisms. Furthermore, the additive level of EPSCs produced by a combination of DTX and nicotinic receptor stimulation (Fig. 6) substantially exceeds that of the DTX and 5-HT combination, demonstrating that occlusion of 5-HT by DTX is not attributable to a physiological or measurement ceiling.

**Evidence for involvement of another channel**

Preliminary evidence suggests that adding a low concentration of TEA together with DTX further occludes the 5-HT effect, as illustrated in Figure 7. Spiking-associated blockade of channels containing Kv1.2 by DTX would normally activate high-voltage- or calcium-activated potassium channels, tending to limit the period of terminal depolarization. If 5-HT blocked one or more such currents in addition to the DTX-sensitive current, it would account for the larger effect of 5-HT on glutamate release. Two possible candidates are Kv3.2, a high-voltage-activated current, and BK, a calcium- and voltage-activated conductance (see results). Additional work is necessary to explore these possibilities.

**Presynaptic versus postsynaptic location of 5-HT_2A receptors**

5-HT_2A receptor mRNA is heavily expressed in cerebral cortex (Mengod et al., 1990). There is a dense band of 5-HT_2A receptor binding in superficial layer V of prefrontal cortex (Blue et al., 1988). Immunohistochemical studies have shown a high density of 5-HT_2A receptors in apical dendrites of layer V pyramidal neurons (Willins et al., 1997; Hamada et al., 1998) and especially in postsynaptic densities of asymmetric terminals on apical dendrites (Hamada et al., 1998). In contrast, there is sparse 5-HT_2A receptor expression in thalamus (Mengod et al., 1990), and electron microscopy shows little or no 5-HT_2A immunoreactivity in cortical nerve terminals (Hamada et al., 1998; Jakab and Goldman-Rakic, 1998; Cornea-Hebert et al., 1999).

We showed that blockade of Kv1.2-containing voltage-dependent potassium channels by DTX markedly occludes 5-HT-induced EPSCs. Models depicting two possible mechanisms for 5-HT_2A-mediated inhibition of Kv1.2 are shown in Figure 8 (another model is suggested by Scruggs et al., 2000). The first model is based on the assumption of a presynaptic location for the 5-HT_2A receptor in which, for example, activation of the G_q-coupled receptor could lead to phosphorylation and inhibition of

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**Figure 8.** Models depicting two possible mechanisms for 5-HT_2A-mediated inhibition of Kv1.2-containing potassium channels, leading to TTX-sensitive glutamate release from thalamocortical terminals. As discussed previously (Aghajanian and Marek, 1999b), the 5-HT_2A receptors responsible for inducing EPSCs could be located presynaptically or postsynaptically. A. A presynaptic model suggests that the G_q-coupled receptor activates an intracellular pathway capable of inhibiting both the Kv1.2-containing low-voltage-activated channel and a high-voltage- or calcium-activated potassium channel. This model could be either direct (as shown here) or indirect through an intervening excitatory interneuron, as suggested by Scruggs et al. (2000). B. A postsynaptic model suggests the ability of the G_q-coupled 5-HT_2A receptor to release a retrograde messenger capable of bringing about blockade or inhibition of the aforementioned potassium channels. Additional work is necessary to explore these possibilities.

Cortex is heavy in the neuropil adjacent to layer V apical dendrites, yet conspicuously absent from the cell bodies. These studies do not agree as to whether there is Kv1.2 expression in layer V cortical neurons (Sheng et al., 1994; Wang et al., 1994). There is, however, a high level of Kv1.2 expression in thalamus (Kues and Wunder, 1992), which is congruent with our results that DTX induces glutamate release from a population of thalamocortical terminals but does not consistently depolarize cortical neurons (also shown by Bekkers and Delaney, 2001). However, it is also possible that Kv1.2-containing channels are both presynaptic and postsynaptic, and differences in the type or stoichiometry of the other subunits in the channels render these populations differentially sensitive to DTX. Several different combinations containing Kv1.2 subunits, including Kv1.2 homomers and several different heteromultimers, have been shown in homogenates of bovine and human cortex using sequential immunoprecipitation with specific Kv subunit antibodies (Shamotienko et al., 1997; Coleman et al., 1999; Wang et al., 1999). However, these studies cannot address
Kv1.2. The latter could be through the activation of a tyrosine kinase, as has been shown for G αi-activated Pyk2 in expression systems (Huang et al., 1993; Imbrić et al., 2000). However, recent work showing a dramatic upregulation of 5-HT 2A receptor immunostaining in the weeks after lesions of midline thalamus (Marek et al., 2001) makes a presynaptic mechanism appear unlikely. The second model in Figure 7 shows 5-HT 2A receptors located postsynaptically on layer V apical dendrites and suggests that their activation may release a retrograde messenger that could interact with certain voltage-gated potassium channels on presynaptic terminals. For example, 5-HT 2A receptors have been shown to stimulate phospholipase A 2 (Felder et al., 1990; Kurrasch-Orbaugh et al., 2000), leading to the formation of arachidonic acid, which is an extracellular blocker of Kv1.2 homomers in expression systems and at least one member of the high-voltage-activated Kv3 family (Poling et al., 1995, 1996).

**Cognitive and clinical implications**

High-frequency stimulation of cortical projections from midline and intralaminar nuclei of thalamus results in a long-lasting increase in cortical arousal (Dempsey and Morison, 1942; Groenewegen and Berendse, 1994); similarly, 5-HT 2A receptor stimulation promotes cortical arousal (Vollenweider et al., 1997; Hermle et al., 1998). During waking, 5-HT levels are five times higher than during slow-wave sleep (de Saint Hilaire et al., 2000). Increasing 5-HT at the synapse with selective serotonin reuptake inhibitors frequently results in decreases in quantity and quality of sleep, which can be prevented by cotreatment with a 5-HT 2A receptor antagonist (Oberndorfer et al., 2000). Animals placed in novel situations show increases in cortical 5-HT (Reuter and Jacobs, 1996). In humans, selective stimulation of 5-HT 2A receptors result in hyperexcitation of frontal cortex and can produce hallucinations (Vollenweider et al., 1997, 1998).

The ability of voltage-gated potassium channel blockers to mimic 5-HT 2A activation suggests that subepileptic levels of potassium channel block may also cause increases in cortical arousal, similar to that caused by 5-HT 2A agonists. In humans, 4-AP has been shown to accelerate waking after anesthesia (Sta et al., 1982). Early reports of DTX administration to mice report hypersensitivity and hyper-reactivity to sound and touch (Harvey and Karlsson, 1980; Silveira et al., 1988). Similarly, administration of low doses of 4-AP to horses report excitation and exaggerated responses to external stimuli (Klein and Hopkins, 1981).

In this study, we showed striking parallels in modulation of glutamate release from thalamocortical terminals by either 5-HT 2A receptor stimulation or Kv1.2 voltage-gated potassium channel block. A review of the literature shows surprising similarities and interactions in the effects of voltage-gated potassium channel blockers, 5-HT 2A receptor agonists, and midline thalamic stimulation. Insight into the cellular mechanisms through which 5-HT 2A receptor agonists alter cortical arousal and information processing may provide clues about mechanisms underlying normal cortical arousal and perturbations that occur in psychosis.

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