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Actions of dopamine and dopaminergic drugs on cloned serotonin receptors expressed in *Xenopus* oocytes

(dopamine receptors/fenoldopam/inositol 1,4,5-trisphosphate/pharmacology/SCH 23390)

R. M. WOODWARD, M. M. PANICKER, AND R. MILEDI

Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine, CA 92717-4550

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ABSTRACT **Using electrophysiological techniques, we studied interactions of dopamine and selected dopaminergic drugs with serotonin (5-hydroxytryptamine; 5-HT) receptors expressed in *Xenopus* oocytes by RNAs transcribed from cloned cDNAs. Oocytes showing strong expression of 5-HT_{1c} and 5-HT_2 receptors became weakly responsive to the neurotransmitter dopamine, which, like 5-HT, elicited Cl^- currents through activation of the phosphatidylinositol/Ca^{2+} messenger pathway. The two types of 5-HT receptors showed similar sensitivity to dopamine; threshold responses were activated at concentrations as low as 1 μM. However, maximum dopamine responses were only 5–20% of maximum responses activated by 5-HT. The dopamine D1 receptor antagonist SCH 23390 was a potent agonist on 5-HT_{1c} and 5-HT_2 receptors. SCH 23390 elicited currents at concentrations as low as 1 nM, but maximum responses were again only 5–20% of those activated by 5-HT. Fenoldopam, a dopamine D1 receptor agonist, also interacted with 5-HT_{1c} and 5-HT_2 receptors, eliciting threshold responses between 10 and 20 nM. Our experiments raise the possibility that low micromolar concentrations of dopamine can cause weak activation and concomitant desensitization of serotoninergic systems *in vivo* and demonstrate that benzazepines can interact with 5-HT receptors at nanomolar concentrations.**

Serotoninergic systems are widespread in mammalian brain, and their dysfunction has been implicated in a variety of neurological disorders (e.g., refs. 1 and 2). Serotonin (5-hydroxytryptamine; 5-HT) has been shown to act through numerous receptor subtypes, which show differential patterns of expression in brain and are coupled to different ion channels and intracellular messenger pathways (e.g., ref. 3).

Early studies indicated that poly(A)$^+$ RNA extracted from rat or human brain expressed functional 5-HT receptors in *Xenopus* oocytes, thus providing an additional approach for investigations into the molecular biology and pharmacology of these proteins (4). When assayed by electrical recording techniques, exogenous 5-HT receptors were found to mediate at least three membrane current responses in oocytes: two involving increases in membrane conductance to Cl^- and one involving a reduction in conductance to K^+ (4–6). The dominant electrical response was elicited through activation of an endogenous intracellular messenger pathway, which was triggered by receptor-stimulated hydrolysis of inositol phospholipids, liberation of intracellular Ca^{2+} by inositol polyphosphates, and activation of endogenous Ca^{2+}-gated Cl^- channels in the oocyte membrane (7, 8). In addition, inositol 1,4,5-trisphosphate was found to regulate Ca^{2+} channels in the plasma membrane, stimulating entry of extracellular Ca^{2+} and further activation of Cl^- currents (9). The oocyte expression system was subsequently exploited in "expression-cloning" procedures used to isolate clones encoding 5-HT_{1c} receptors from mouse and rat choroid plexus cDNA libraries (10, 11). Sequence data from the 5-HT_{1c} receptor clone was then used to isolate cDNAs encoding the 5-HT_2 receptor from rat cerebral cortex (12, 13).

In the present study, effects of dopamine and selected dopaminergic drugs were assayed on rat 5-HT_{1c} and 5-HT_2 receptors expressed in *Xenopus* oocytes by RNA transcribed from cloned cDNAs. The initial impetus for this work came from the observation that oocytes expressing 5-HT_{1c} receptors not only developed high sensitivity to 5-HT but simultaneously became responsive to the neurotransmitter dopamine.

MATERIALS AND METHODS

Preparation of RNAs and Microinjection of Oocytes. Plasmid pSR1c (11) was linearized with *Not* I and transcribed with T7 RNA polymerase to obtain capped RNA encoding rat 5-HT_{1c} receptors. Capped RNA encoding 5-HT_2 receptors was obtained using T7 RNA polymerase from plasmid pSR2 (13) and was linearized with *Hin*dIII. *Xenopus* oocytes were plucked from the ovary and injected with 0.5–5 ng of RNA transcribed from pSR1c, or 7–70 ng of RNA transcribed from pSR2 (injection volume, 50 nl). RNA concentrations were determined by absorbance at 260 nM. Oocytes were stored in Barth's medium [88 mM NaCl/1 mM KCl/0.33 mM $Ca(NO_3)_2$/0.41 mM $CaCl_2$/0.82 mM $MgSO_4$/2.4 mM $NaHCO_3$/5 mM Hepes, pH 7.4, usually with gentamycin at 0.1 mg/ml]. Two days after injection, oocytes were defolliculated by a 0.75- to 1-hr treatment with collagenase (Sigma type I; 200 units/mg) at 0.5–1.0 mg/ml (14).

Electrophysiology, Pharmacology, and Data Analysis. Membrane current responses were recorded by using a conventional two-electrode voltage clamp, over periods between 3 and 9 days after injection. Recordings were made in a 0.1-ml chamber continuously perfused at 2–10 ml/min with frog Ringer's solution (115 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/5 mM Hepes, pH 7.0). All drugs were applied by bath perfusion. Accurate measurement of 5-HT concentration–response relationships was complicated by desensitization and, in some cases, by what appeared to be "facilitation" between responses (cf. ref. 15). Distortion of concentration–response relationships resulting from desensitization was reduced by using prolonged wash intervals between responses (up to 3 hr in some cases). Membrane current responses were normalized and expressed as a fraction of the maximum peak response. EC_{50} values were calculated from concentration–response curves using a nonlinear least-squares curve-fitting program, based on a four-parameter logistic equation (16), and IC_{50} values were determined by regression.

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); T_{in} current, transient inward Cl^- current(s) activated upon hyperpolarizing steps in potential.

Drugs. Dopamine and 5-HT were from Sigma or Research Biochemicals (Natick, MA); 1–10 mM stocks of dopamine were made up in Ringer's solution 2–5 min before dilution and use. (*S*)-(−)-Eticlopride, (−)-quinpirole (LY 171555), (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hydrochloride (SCH 23390), the (*S*)-(−) stereoisomer of SCH 23390 (SCH 23388), and (*R*)-(+)-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride [(*R*)-(+)-SKF 38393] were all from Research Biochemicals and were made up daily as 0.1 mM stocks in Ringer's solution. Fenolodopam (SKF 82526) was provided by Smith Kline & French. Spiperone (spiroperidol) was from Research Biochemicals and was made up as 0.1, 1, and 10 mM stocks in dimethyl sulfoxide.

RESULTS

Expression of 5-HT_{1c} and 5-HT_2 Receptors in *Xenopus* Oocytes. Injection of 0.5–5 ng of RNA transcribed from pSR1c resulted in strong expression of 5-HT_{1c} receptors, whereas strong expression of 5-HT_2 receptors typically required injection of 70 ng of RNA transcribed from pSR2. In oocytes showing high levels of responsiveness, concentrations of 5-HT necessary to elicit threshold membrane current responses were as low as 0.3–1 nM for either type of 5-HT receptor. This apparent similarity in sensitivity was somewhat surprising as 5-HT_2 receptors had originally been characterized as having comparatively low affinity for 5-HT (e.g., ref. 3). The pharmacological integrity of the receptors expressed in oocytes was therefore rechecked, to the extent of assaying sensitivity to spiperone. The IC_{50} for spiperone on 5-HT_2 receptors was 1.6 ± 0.5 nM (n = 3, data given as mean ± SD), whereas the IC_{50} on 5-HT_{1c} receptors was 15 ± 6 μM (n = 3), ≈10,000 times higher (inhibition measured on currents elicited by 100 nM and 10 nM 5-HT, respectively).

For both types of 5-HT receptors, maximum currents were activated by 1–10 μM 5-HT and ranged between 4 and 15 μA in different oocytes (holding potential of −60 mV). EC_{50} values for 5-HT_{1c} and 5-HT_2 receptors were 51 ± 30 nM 5-HT (n = 5) (Fig. 1*B*) and 170 ± 55 nM 5-HT (n = 3), respectively. The variation in size of maximum responses reflected not only variations in numbers of receptors expressed but also saturation of some element of the endogenous intracellular messenger system. It was therefore likely that concentration–response curves measured in oocytes showing high levels of expression were not an accurate reflection of receptor occupancy.

Actions of Dopamine on 5-HT_{1c} and 5-HT_2 Receptors Expressed in Oocytes. Oocytes showing strong expression of 5-HT_{1c} or 5-HT_2 receptors were assayed for responsiveness to catecholamines, and whereas epinephrine and norepinephrine (1–100 μM) did not evoke appreciable membrane currents, micromolar concentrations of dopamine elicited substantial responses (Fig. 1*A*). The membrane currents elicited by dopamine were inward at holding potentials of −60 mV and showed similar latency, biphasic time course, and current oscillations as those elicited by 5-HT, all of which were characteristic of responses mediated by the endogenous phosphatidylinositol/Ca^{2+} messenger pathway. The voltage dependence of dopamine responses was determined, and this confirmed that the current (*i*) reversed between −20 and −30 mV, corresponding to the equilibrium potential for Cl^- (17); and (*ii*) rectified strongly at negative potentials, a property of Ca^{2+}-gated Cl^- channels (Fig. 1*C*) (8). Moreover, dopamine responses were typically associated with transient inward Cl^- currents activated upon hyperpolarizing steps in potential (T_{in}) and were abolished after chelation of intracellular Ca^{2+} by intraoocyte injections of 50–100 pmol of EGTA (5, 8, 9). These experiments all served to confirm that responses activated by dopamine in oocytes expressing 5-HT_{1c} or 5-HT_2 receptors were mediated by the phosphatidylinositol/Ca^{2+} receptor-channel coupling system.

Levels of responsiveness to dopamine invariably paralleled those to 5-HT; thus oocytes showing low levels of 5-HT receptor expression gave correspondingly small responses to dopamine. Uninjected oocytes from the same frogs did not respond to either 5-HT or dopamine. Furthermore, responses elicited by 5-HT and dopamine were reduced or abolished in parallel by antagonists. For example, in oocytes expressing 5-HT_{1c} receptors, currents generated by both agonists showed only low sensitivity to spiperone (IC_{50} between 10 and 20 μM) but relatively high sensitivity to mianserin (IC_{50} between 100 and 500 nM). In oocytes expressing 5-HT_2 receptors, responses elicited by either neurotransmitter had high sensitivity to spiperone (IC_{50} between 1 and 2 nM). Taken together, these results indicated that dopamine elicited currents through direct interactions with 5-HT_{1c} and 5-HT_2 receptors.

In oocytes showing high levels of 5-HT_{1c} or 5-HT_2 receptor expression, dopamine activated threshold responses at concentrations ranging between 1 and 3 μM (Fig. 2*A*). Concentration–response relationships for both subtypes of the 5-HT receptor showed that the EC_{50} for dopamine-activated currents ranged between 50 and 100 μM. But more importantly, maximum responses, elicited by 1–5 mM dopamine, were only 5–20% of the maximum currents elicited by 5-HT (Fig. 1*B*). Simultaneous application of dopamine together with

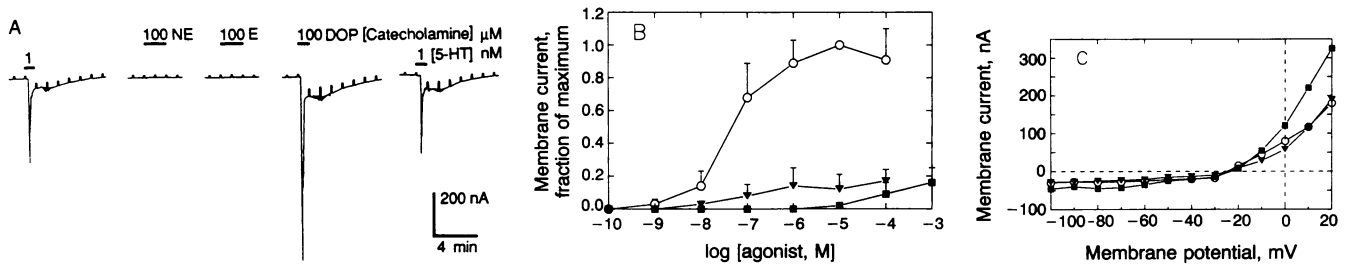

FIG. 1. (*A*) Membrane current responses elicited by 5-HT and different catecholamines in the same oocyte expressing 5-HT_{1c} receptors. NE, norepinephrine; E, epinephrine; DOP, dopamine. To minimize effects of receptor desensitization, individual records were separated by 10–40 min, depending on the size of preceding response. Unless otherwise stated, the holding potential in this and all following records was −60 mV, stepped at 1-min intervals to −50 mV for 7–8 s (small upward deflections) to monitor membrane conductance; drug applications are indicated by bars. The perfusion dead time was 5–15 s. Inward currents correspond to downward deflection. Capacitative transients on steps in potential were deleted during preparation of figures. (*B*) Concentration–response curves comparing currents elicited by 5-HT (○), dopamine (■), and SCH 23390 (▼) through activation of 5-HT_{1c} receptors expressed in oocytes. Data points are the mean ± SD (n = 4–6) expressed as a fraction of the current elicited by 10 μM 5-HT (maximum response). (*C*) Voltage dependence of currents elicited by 1 nM 5-HT (○), 50 μM dopamine (■), and 50 nM SCH 23390 (▼) in an oocyte expressing 5-HT_{1c} receptors. The membrane current was measured by briefly stepping to different potentials during relatively sustained responses and then subtracting currents found in the unstimulated membrane. Inward current is denoted by negative nanoamperes.

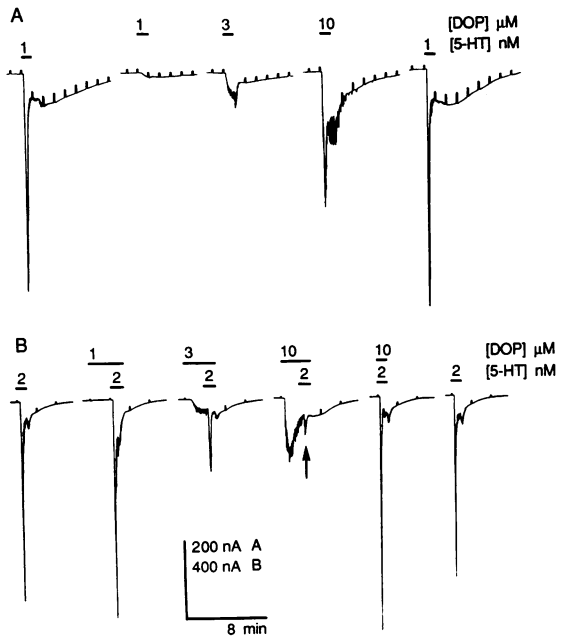

FIG. 2. (*A*) Assay measuring the concentration of dopamine (DOP) necessary to elicit threshold membrane current responses in an oocyte expressing 5-HT_2 receptors. Records were separated by a 15–30 min wash. (*B*) Interactions between responses activated by 5-HT and dopamine in an oocyte expressing 5-HT_{1c} receptors. First record, control 5-HT response. Second record, preincubation in 1 μM dopamine caused no inhibition of the 5-HT response. Third record, preincubation in 3 μM dopamine elicited a small oscillatory current and suppressed the subsequent 5-HT response by 65%. Fourth record, preincubation in 10 μM dopamine elicited an oscillatory current that peaked at 325 nA and decayed to <100 nA; the subsequent 5-HT response (arrow) was suppressed by ≈90%. Fifth record, simultaneous application of 5-HT and 10 μM dopamine caused no clear inhibition of the 5-HT response. Sixth record, control. Records were separated by a 30–40 min wash.

5-HT caused modest reductions in 5-HT responses, suggesting dopamine was a partial agonist of both types of 5-HT receptor. For example, in oocytes expressing 5-HT_{1c} receptors, currents elicited by 100 nM 5-HT were reduced 37% ± 22% by 1 mM dopamine ($n = 6$). This type of experiment also appeared to rule out the possibility that responses activated by dopamine were simply due to low-level contamination by 5-HT. For instance, if dopamine responses were due to contaminating 5-HT, then 1 mM dopamine should have elicited currents that were 50–80% of the maximum 5-HT responses, instead of the 5–20% recorded.

Though direct inhibitory actions of dopamine on 5-HT_{1c} and 5-HT_2 receptors were weak, "indirect" inhibition of 5-HT responses, due to receptor desensitization, was relatively strong. For example, currents elicited by 1–10 nM 5-HT were reduced 70–90% following 2-min preincubations in low micromolar concentrations of dopamine, sufficient to elicit small membrane current responses (Fig. 2*B*). In contrast, the same 5-HT responses were either unaffected or marginally facilitated by 2-min preincubations in subthreshold concentrations of dopamine or simultaneous application with 10 μM dopamine (Fig. 2*B*).

Actions of Dopaminergic Drugs on 5-HT_{1c} and 5-HT_2 Receptors Expressed in Oocytes. Interactions of dopamine with 5-HT receptors prompted us to utilize the oocyte system to assay effects of various dopaminergic drugs on rat 5-HT_{1c} and 5-HT_2 receptors; the studies were focused on drugs that showed a large degree of specificity between different subtypes of dopamine receptors.

SCH 23390 (Dopamine D1 Receptor Antagonist). When oocytes with high levels of 5-HT_{1c} or 5-HT_2 receptor expression were assayed, SCH 23390 was itself potent in eliciting membrane current responses. Concentrations of SCH 23390 necessary to elicit threshold responses were as low as 1–3 nM for both types of 5-HT receptors, only 3–10 times higher than thresholds for 5-HT in the same oocytes. SCH 23390 responses again showed the characteristic oscillatory time course and voltage dependence of responses elicited through activation of the phosphatidylinositol/Ca^{2+} pathway (Fig. 1*C*). As described for dopamine, the sensitivity of oocytes to SCH 23390 clearly paralleled that to 5-HT, and uninjected oocytes gave no oscillatory response. In oocytes expressing 5-HT_2 receptors, currents activated by SCH 23390 were effectively abolished by 10 nM spiperone, whereas SCH 23390 responses in oocytes expressing 5-HT_{1c} receptors were only weakly sensitive to spiperone but were strongly inhibited by mianserin (500 nM).

These experiments all implied that SCH 23390 responses were elicited through direct interactions with 5-HT receptors. However, comparisons of currents elicited by SCH 23390 and 5-HT in the same oocyte indicated that the time courses of the two responses were different. One-minute applications of 5-HT (1–100 nM) elicited the characteristic transient "spike" of oscillatory Cl^- current, followed by a smooth component, upon which small oscillatory currents were superimposed. The smooth component normally washed out within <10 min and was associated with clear T_{in} currents (Fig. 3*A*). In contrast, SCH 23390 responses typically had longer latency, followed a slower time course, and were washed out relatively slowly. For example, even when using low concentrations of SCH 23390 (5–10 nM), responses commonly required at least 30 min to be fully washed out and were associated with correspondingly prolonged activation of T_{in} currents (Fig. 3*B*). Spikes of oscillatory current, which characterized 5-HT and dopamine responses, were only elicited when SCH 23390 was applied at higher concentrations (50–100 nM).

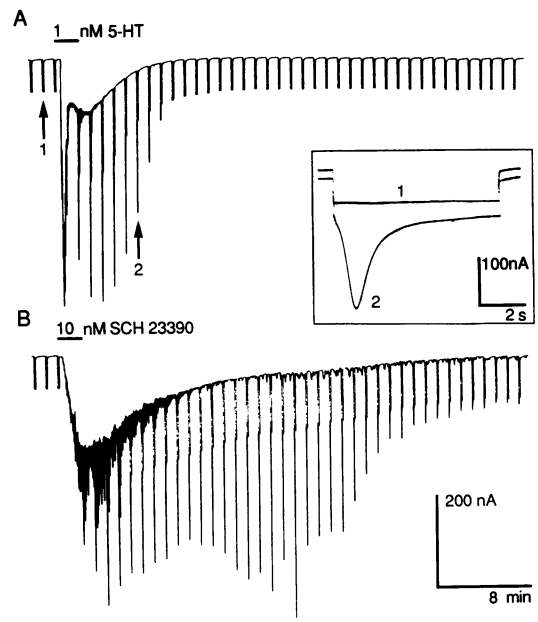

FIG. 3. Membrane current responses elicited by 5-HT and SCH 23390 in an oocyte expressing 5-HT_2 receptors. (*A*) Response elicited by a 2-min application of 1 nM 5-HT. The holding potential was −60 mV, pulsed for 7 s to −110 mV at 1-min intervals. (*Inset*) Sample current traces of two voltage steps (arrows) recorded using an expanded time scale. Arrow 1, current pulse prior to application of 5-HT; arrow 2, T_{in} current activated after a 5-min wash of 5-HT. (*B*) Prolonged response elicited by a 2-min application of 10 nM SCH 23390, illustrating extended activation of T_{in} currents.

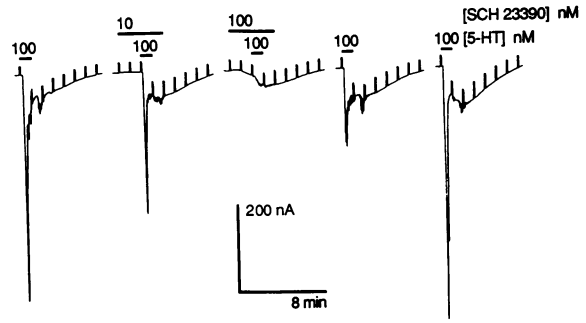

FIG. 4. Inhibitory effects of SCH 23390 on currents elicited by 5-HT in an oocyte expressing 5-HT_2 receptors. This oocyte was considerably less sensitive to 5-HT and SCH 23390 than the oocyte in Fig. 3. First record, control 5-HT response. Second record, SCH 23390 at 10 nM did not activate membrane currents but inhibited the 5-HT response. Third record, SCH 23390 at 100 nM activated a small current (latency of 1 min) and inhibited the 5-HT response by >90%. Fourth record, inhibition caused by 100 nM SCH 23390 was refractory to a 40-min wash. Fifth record, return of control response following a >1-hr wash. Records were separated by intervals of 40 min.

Concentration–response relationships showed that the maximum currents elicited by SCH 23390 were only 10–25% of maximum responses elicited by 5-HT (Fig. 1*B*). Moreover, for both types of 5-HT receptors, SCH 23390 appeared to have inhibitory actions on 5-HT responses that were independent of effects due to desensitization of receptors. For instance, using oocytes with slightly lower levels of responsiveness to 5-HT, 10 nM SCH 23390 elicited no detectable current during 2-min preincubations but, nonetheless, suppressed 5-HT responses by between 40% and 60%. Inhibitory effects of SCH 23390 were reversible, but again washed out slowly, often requiring >1 hr before 5-HT responses returned to control levels (Fig. 4). SCH 23390 was at least 1000 times more active than SCH 23388 [the (*S*)-(−)-enantiomer] in eliciting membrane currents, indicating high levels of stereoselectivity in the agonist activity.

Fenolodopam and (*R*)-(+)-SKF 38393 (Dopamine D1 Receptor Agonists). As described for SCH 23390, fenoldopam (SKF 82526) and (*R*)-(+)-SKF 38393 were assayed for interactions with 5-HT_{1c} and 5-HT_2 receptors by using oocytes showing high levels of receptor expression. Both agonists were potent in activating membrane current responses mediated by the phosphatidylinositol/Ca^{2+} pathway (Fig. 5*B*). For both types of 5-HT receptors, threshold responses were activated between 10 and 50 nM. Responses elicited by fenoldopam or (*R*)-(+)-SKF 38393 in oocytes expressing 5-HT_2 receptors were again strongly inhibited by 10 nM spiperone, whereas responses elicited in oocytes expressing 5-HT_{1c} receptors were only weakly sensitive to spiperone but were inhibited by

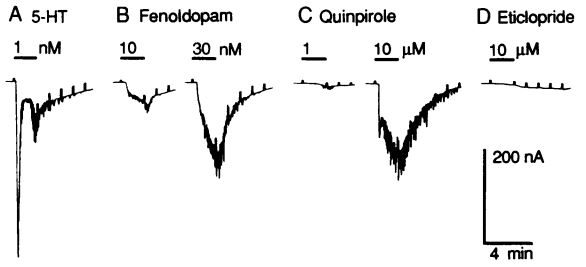

FIG. 5. Relative agonist activities of dopaminergic drugs in a single oocyte expressing 5-HT_2 receptors. (*A*) Control 5-HT response. (*B*) Currents activated by fenoldopam. (*C*) Currents activated by (−)-quinpirole. (*D*) Marginal response elicited by (*S*)-(−)-eticlopride. Reproducible control 5-HT responses were repeated after *B* and *C* (not shown); records were separated by 20–60 min.

mianserin. Fenoldopam responses, together with associated T_{in} currents, washed out relatively rapidly, and apart from effects that could be attributed to desensitization of receptors, fenoldopam (30–50 nM) had no clear blocking effects on currents elicited by 1 nM 5-HT. On the other hand, time courses of (*R*)-(+)-SKF 38393 responses were slow, were associated with prolonged activation of T_{in} currents, and were refractory to washing, similar to responses activated by SCH 23390 in the same oocytes (data not shown). (*R*)-(+)-SKF 38393 (50–100 nM) also appeared to have distinct inhibitory effects on currents elicited by 1–10 nM 5-HT.

Quinpirole (Dopamine D2 Receptor Agonist). (−)-Quinpirole was less active than any of the dopamine D1-selective drugs assayed in this study but likewise elicited the characteristic Cl^- responses in oocytes expressing either 5-HT_{1c} or 5-HT_2 receptors (Fig. 5*C*). Concentrations of (−)-quinpirole necessary to activate threshold responses were between 1 and 3 μM for both types of 5-HT receptors, and time courses of responses were similar to those described for 5-HT, dopamine, and fenoldopam. At concentrations up to 10 μM, simultaneous application of quinpirole had no clear inhibitory effects on currents elicited by 1 nM 5-HT.

Eticlopride (Dopamine D2 Receptor Antagonist). (*S*)-(−)-Eticlopride was largely inactive as an agonist of 5-HT_{1c} and 5-HT_2 receptors. In some oocytes showing strong expression of 5-HT_2 receptors, 10 μM (*S*)-(−)-eticlopride appeared to activate marginal, prolonged inward currents (e.g., Fig. 5*D*), but these effects were inconsistent. For both types of 5-HT receptor, (*S*)-(−)-eticlopride reversibly suppressed currents elicited by 10–100 nM 5-HT ($IC_{50} \approx 1$ μM).

DISCUSSION

Our experiments indicate that the neurotransmitter dopamine has weak, but significant, agonist activity on cloned rat 5-HT_{1c} and 5-HT_2 receptors expressed in *Xenopus* oocytes. Dopamine was ≈10,000 times less potent than 5-HT in terms of threshold responses and activated maximum responses that were only 5–20% of those elicited by 5-HT. Nonetheless, effects on both 5-HT_{1c} and 5-HT_2 receptors were apparent at concentrations as low as 1 μM dopamine. The weak agonist activity on 5-HT receptors implies that the net effect of dopamine on serotoninergic receptor systems should be low-level stimulation. However, our results suggest that low micromolar concentrations of dopamine interacting with serotoninergic systems *in vivo* could be predominantly inhibitory, primarily through stimulating desensitization of 5-HT receptors.

5-HT_{1c} and 5-HT_2 receptors showed little or no differences in their responsiveness to dopamine, or indeed, to any of the dopaminergic drugs assayed in this study. At the molecular level, this suggests that sensitivity to dopamine involves structural features that are common to both types of 5-HT receptors and that are also represented in dopamine receptors. To date, at least six different subtypes of mammalian dopamine receptors have been cloned; all belong to a superfamily of receptors that couple to GTP-binding proteins and are characterized by seven putative membrane-spanning regions (e.g., refs. 18–22). Dopamine receptors have two serine residues conserved in the fifth transmembrane region, which appear to be important for agonist binding and receptor activation, and a conserved aspartate residue in the third transmembrane region, which is believed to be involved in binding cationic amines (22–24). 5-HT1c and 5-HT_2 receptors have 51% homology to each other and are homologous to dopamine receptors (37% homology with the dopamine D1a subtype). In particular, the fifth transmembrane region in both 5-HT receptors is identical, and interestingly, one of the serine residues critical for agonist binding in dopamine receptors is conserved (Ser-220 in 5-HT_{1c} and Ser-217 in 5-HT_2)

(11, 12, 22–25). Furthermore, the aspartate residue in the third transmembrane region of dopamine receptors is also conserved in the two 5-HT receptors (Asp-135 in 5-HT_{1c} and Asp-133 in 5-HT_2) (11, 12). These common features, in what is believed to define agonist binding sites in dopamine and 5-HT receptors, might provide an explanation at the molecular level for the observed sensitivity of 5-HT_{1c} and 5-HT_2 receptors to dopamine and hence to some dopaminergic drugs.

Actions of dopamine on 5-$HT1_c$ and 5-HT_2 receptors were only pronounced in oocytes showing high levels of receptor expression, and determining whether there is any significance to this dual sensitivity in the functioning of mammalian nervous systems, under normal or pathological conditions, will clearly require *in vivo* studies. At present it even remains possible that sensitivity of 5-HT receptors to dopamine is for some reason specific to receptors expressed in oocytes—perhaps due to the foreign lipid environment or to a degree of infidelity in the endogenous signal transduction pathway (e.g., rat receptors coupling to *Xenopus* GTP-binding proteins), which leads to activation of responses that would not normally be elicited in mammalian tissues. However, clear correspondences between the effects of dopaminergic drugs on 5-HT receptors expressed in oocytes, and effects of these drugs on 5-HT receptors in mammalian brain, argue that dopamine likewise interacts with 5-HT receptors *in situ*.

The benzazepine derivative SCH 23390 was initially characterized as a selective antagonist on dopamine D1 receptors (26), but subsequent binding studies suggested interactions with 5-HT_1 and 5-HT_2 receptors in rat brain (e.g., ref. 27). In particular, SCH 23390 was shown to bind 5-HT_{1c} receptors in the choroid plexus (28) and, moreover, to decrease production of cerebrospinal fluid (29). Our results show that SCH 23390 potently activated cloned rat 5-HT_{1c} and 5-HT_2 receptors expressed in oocytes, eliciting membrane current responses at concentrations as low as 1 nM. In addition, concentration–response curves, and the clear inhibitory effects on currents elicited by 5-HT, suggested that SCH 23390 acted as a partial agonist/antagonist on these receptors. As described for dopamine, the complex stimulatory and inhibitory effects of SCH 23390 on 5-HT_{1c} and 5-HT_2 receptors would appear to make it difficult to predict the predominant effects of this drug on serotoninergic systems *in vivo*. Fenoldopam and (*R*)-(+)-SKF 38393 are also benzazepine derivatives, closely related to SCH 23390, and both have been shown to have selective agonist activity on dopamine D1 receptors in rat brain (e.g., ref. 30). Recent studies on rat fundus muscle and the cardiovascular system have suggested that fenoldopam, or SKF 87516 (a fluoro analogue), also interacts with 5-HT receptors (31, 32). Our experiments showed that both drugs had agonist activity on 5-HT_{1c} and 5-HT_2 receptors expressed in oocytes; fenoldopam elicited responses on 5-HT_2 receptors at concentrations as low as 10 nM.

Overall, our results confirm that a variety of dopaminergic drugs interact with 5-HT_{1c} and 5-HT_2 receptors and show how the oocyte expression system can be fruitfully used in this type of pharmacological characterization. In the case of the benzazepine derivatives SCH 23390, SKF 38393, and fenoldopam, effects on 5-HT_{1c} and 5-HT_2 receptors appear to be sufficiently potent to warrant the need for appropriate controls, wherever possible, and some caution in interpretation of results.

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