Activation of the 5-HT_{1C} receptor expressed in *Xenopus* oocytes by the benzazepines SCH 23390 and SKF 38393

¹Clark A. Briggs, ‡Nancy J. Pollock, ‡Donald E. Frail, *Cheryl L. Paxson, *Robert F. Rakowski, Chae Hee Kang & John W. Kebabian

Department of Neuroscience and ‡Department of Corporate Molecular Biology, Abbot Laboratories, Abbott Park, Illinois 60064 and *Department of Physiology and Biophysics, The Chicago Medical School, North Chicago, Illinois 60064, U.S.A.

1 A cloned 5-HT_{1C} receptor expressed in *Xenopus laevis* oocytes was used to characterize the action of four dopamine D₁-selective benzazepines at the 5-HT_{1C} receptor. Additionally, the apparent binding of the D₁-selective benzazepines to 5-HT_{1C} receptors was measured in the choroid plexus of the pig.

2 In voltage-clamped oocytes expressing the cloned 5-HT_{1C} receptor, 5-hydroxytryptamine (5-HT) elicited a characteristic inward current response with an EC₅₀ of 13 nM. SCH 23390 acted as a stereoselective agonist (or partial agonist) with an EC₅₀ of about 550 nM. SKF 38393 (1 µM–1 mM), SKF 77434 (100 µM), and SKF 82958 (100 µM) also acted as agonists (or partial agonists) at the cloned 5-HT_{1C} receptor. SKF 38393 was not stereoselective at the 5-HT_{1C} receptor.

3 The response to SCH 23390 activated slowly and, although the response contained many oscillations characteristic of the activation of the phosphatidylinositol signal transduction system, SCH 23390 rarely elicited the rapid spike-like response seen routinely in response to 5-HT. However, the responses to SKF 38393, SKF 77434, and SKF 82958 were identical in appearance to the response to 5-HT, except that the responses to the benzazepines were smaller. These comparisons were made by applying both a benzazepine and 5-HT to each individual oocyte expressing the cloned 5-HT_{1C} receptor.

4 Consistent with the responses measured in oocytes, SCH 23390 bound stereoselectively to 5-HT_{1C} receptors in the choroid plexus of the pig ($K_i = 6.3$ nM), and SKF 38393 bound non-stereoselectively with lower affinity ($K_i = 2.0$ – 2.2 µM).

5 It is concluded that while these benzazepines demonstrate selectivity for the dopamine D₁ receptor, they also can act as agonists or partial agonists at the 5-HT_{1C} receptor *in situ* and as expressed in *Xenopus* oocytes. The oocyte expression system is useful for studies of the functional pharmacology of these 5-HT_{1C} receptors. Information about the pharmacological actions and variations in stereoselectivity among dopamine and 5-HT receptors should be of interest in modelling the interactions of ligands with these G-protein coupled receptors, and in the testing of such models through receptor mutagenesis.

Keywords: Oocytes; *Xenopus laevis*; choroid plexus; 5-hydroxytryptamine; benzazepines; dopamine receptor; 5-HT_{1C} receptor; dopamine; cloning, molecular; pharmacology; molecular biology

Introduction

Benzazepines such as SKF 38393 and SCH 23390 appear to be selective, although not entirely specific, for the D₁-dopamine receptor, and these compounds have been the standards used in many experiments to define responses mediated by D₁ receptors. However, at higher concentrations these benzazepines also interact with 5-hydroxytryptamine (5-HT) receptors, particularly 5-HT_{1C} and 5-HT₂ (Hicks *et al.*, 1984; Dawson *et al.*, 1986; Hess *et al.*, 1986; Bischoff *et al.*, 1986; 1988; De Keyser *et al.*, 1988; 1989; Hoyer & Karpf, 1988; Nicklaus *et al.*, 1988; Skarsfeldt & Larsen, 1988; Hoyer *et al.*, 1989; Yamamoto & Kebabian, 1989). Indeed, the affinity of SCH 23390 for the 5-HT_{1C} receptor is only about 10–30 fold less than for the D₁ receptor. Thus, in heterogeneous systems with multiple receptors, the effects of the D₁ 'benchmark' compounds SKF 38393 and SCH 23390, especially at micromolar doses, are not necessarily due to the activation and inhibition of dopamine D₁-receptors.

While there have been several publications concerned with the binding of benzazepines at 5-HT_{1C} receptors, there has been less work on the functional consequences of this interaction (see Hoyer *et al.*, 1989). We thought it of value to characterize the action and stereoselectivity of such benzazepines at the 5-HT_{1C} receptor. Are, for example, agonists at the D₁ receptor also agonists at the 5-HT_{1C} receptor? It was also thought that such comparative information would be valuable in the molecular modelling of these receptors and their interactions with ligands. Both the dopamine D₁ and the 5-HT_{1C}

receptors are members of the larger class of G-protein coupled receptors. While D₁ and D₂ dopamine receptors clearly are related, in terms restricted to benzazepine binding the D₁ receptor appears more similar to the 5-HT_{1C} receptor than to the D₂ receptor.

In the brain, recent evidence suggests that 5-HT_{1C} receptors are expressed outside the choroid plexus (Conn & Sanders-Bush, 1985; Hoyer *et al.*, 1986a; Peroutka, 1986; Hoffman & Mezey, 1989; Molineaux *et al.*, 1989; Mengod *et al.*, 1990), raising questions about the role of 5-HT_{1C} receptors in the CNS. However, assessing 5-HT_{1C} function pharmacologically could be difficult because of the presence of other 5-HT and dopamine receptors, the lack of highly selective 5-HT_{1C} agonists and antagonists, and the fact that 5-HT₂ and perhaps D₁ receptors in addition to the 5-HT_{1C} receptor are coupled to inositol phospholipid hydrolysis (Hoyer, 1988; Peroutka, 1988; Felder *et al.*, 1989a,b; Hartig, 1989; Schmidt & Peroutka, 1989; Dyck, 1990; Rubinstein & Hitzemann, 1990; Undie & Friedmann, 1990).

One approach would be to utilize *Xenopus laevis* oocytes where the 5-HT_{1C} receptor can be expressed and coupled to the oocyte's endogenous inositol phospholipid system (Lübbert *et al.*, 1987a,b; Hoyer, 1988; Julius *et al.*, 1988; Hartig, 1989). Responses can be measured electrophysiologically through a multi-step signal transduction pathway involving inositol trisphosphate generation from phosphatidylinositol, release of internal Ca²⁺, and activation of a Ca²⁺-dependent Cl[−] current which is the measured response (for reviews see Sumikawa *et al.*, 1986; Lester, 1988; Snutch, 1988; Sigel, 1990). A significant advantage of this approach, using clonal mRNA, is the ability to deal with an individual

¹ Author for correspondence.

selected receptor, assuming that the protein expressed in the oocyte is similar to the protein expressed *in situ*. Additionally, there is the potential for further studies of the receptor, its interaction with ligands, and its interaction with the signal transduction pathway through site-directed mutagenesis and expression of mutated receptors in oocytes.

This paper describes studies on benzazepine functional pharmacology in oocytes expressing the 5-HT_{1C} receptor. Some of the results have been published in an abstract (Briggs *et al.*, 1990).

Methods

5-HT_{1C} receptor cloning

Oligonucleotides were synthesized that encode sequences which flank the coding region of the rat brain 5-HT_{1C} receptor (Julius *et al.*, 1988). cDNA was synthesized from total brain RNA using reverse transcriptase. The oligonucleotides and the cDNA were then used in a polymerase chain reaction (PCR) to generate a fragment that encoded the complete 5-HT_{1C} coding sequence. The fragment was cloned into M13mp19 and completely sequenced to confirm identity with the known 5-HT_{1C} receptor. Three PCR-generated base changes were corrected by site-directed mutagenesis by use of a kit (Biorad, Richmond, CA, U.S.A.).

5-HT_{1C} receptor mRNA

An oligonucleotide (tgtaatacgactcactatagtaaagcagcgccagt) was synthesized that contained both the promoter sequence for T7 RNA polymerase and sequences complimentary to the M13 'universal primer' site. This oligonucleotide and a second M13 complimentary oligonucleotide (commonly referred to as 'reverse primer') were used to amplify the 5-HT_{1C} insert in M13 by PCR. This amplified DNA contained the coding sequence of the 5-HT_{1C} receptor downstream from the T7 promoter and served as a template for *in vitro* transcription producing the 5-HT_{1C} mRNA. *In vitro* transcription for large scale synthesis was performed according to a protocol provided by Promega, except that a cap analogue was included at the start of the reaction (m7G(5')ppp(5')G, 1 mM final concentration; Pharmacia, Piscataway, New Jersey, U.S.A.). After synthesis, the sample was treated with DNase (Promega, Madison, Wisconsin, U.S.A.) and the mRNA was extracted with phenol:chloroform (1:1), precipitated with ethanol, and resuspended in RNase-free water. A portion was analysed by gel electrophoresis and the quantity was estimated by comparison of the ethidium bromide staining intensity with that of RNA size markers (BRL, Gaithersburg, Maryland, U.S.A.).

Oocyte preparation and RNA injection

Oocyte-positive *Xenopus laevis* frogs were obtained from Nasco (Fort Atkinson, WI, U.S.A.) or Xenopus-1 (Ann Arbor, MI, U.S.A.), fed a commercial diet thrice weekly (Nasco or Carolina Biological Supply, Burlington, North Carolina, U.S.A.), and maintained in recirculating, filtered, dechlorinated water at 19–20°C under controlled, full-spectrum lighting (Vita-lite, on 14 h daily). Ovaries were removed from animals after anaesthesia in ice and decapitation followed by spinal pithing, and the oocytes were freed after digestion with collagenase (Sigma Type 1A, 2–2.5 mg ml⁻¹) for 1.5–2.0 h in a low-Ca²⁺ solution (composition mM: NaCl 87.5, KCl 2.5, MgCl 1.0, sodium HEPES buffer 10, pH 7.6) at room temperature (23–25°C). The preparation was washed with the low-Ca²⁺ solution followed by the culture solution (see below), and Stage V-VI oocytes (Dumont, 1972) were selected and maintained for 1–5 days at 15–17°C. In most experiments the culture solution was modified Leibovitz L-15 culture medium, diluted to 67% and supplemented with 2.4 mM NaHCO₃ and 10 mM sodium HEPES buffer (pH 7.55, 233–242 mOsm). In other experiments, the oocytes were maintained in a modified Barth's solution containing (mM) NaCl

90, KCl 1.0, NaNO₃ 0.66, CaCl₂ 0.74, MgCl₂ 0.82, NaHCO₃ 2.4, sodium HEPES buffer 10, and sodium pyruvate 2.5 (pH 7.55, 196–205 mOsm). These solutions also contained antibiotics (100 u ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin) and were sterilized by 0.2 µm filtration. The culture solution was changed daily and any shrunken or mottled oocytes were discarded.

The 5-HT_{1C} mRNA dissolved in RNase-free water was injected by pneumatic pressure into the selected Stage V-VI oocytes within 24 h of their preparation. Typically 10–50 nl was injected per oocyte; the injection volume was estimated by monitoring the injection micropipette with a calibrated video system (see also Smith *et al.*, 1987). As a control, some oocytes were injected with a sample of the RNase-free water.

Response measurement and analysis

Oocytes were used 1–4 days after injection and, during the experiment, were superfused at 2 ml min⁻¹ in a chamber of approximately 550 µl volume. In experiments using oocytes cultured in the modified Barth's solution, the superfusate also was modified Barth's solution (composition mM: NaCl 90, KCl 1.0, NaNO₃ 0.66, CaCl₂ 0.74, MgCl₂ 0.82, NaHCO₃ 2.4, sodium HEPES buffer 10, and sodium pyruvate 2.5, pH 7.55; 196–205 mOsm). In most experiments, however, the oocytes were cultured in the higher-osmolality modified L-15 medium, and recordings were obtained in a modified Ringer solution of similar osmolality (mM: NaCl 110, KCl 2.0, CaCl₂ 1.8, NaHCO₃ 2.4, sodium HEPES buffer 10, and sodium pyruvate 2.5; pH 7.55; 233–242 mOsm).

Each oocyte was voltage-clamped at –70 mV with two intracellular electrodes and a virtual ground circuit (Axoclamp-2A, Axon Instruments Inc., Foster City, CA, U.S.A.). The intracellular electrodes in some initial experiments were filled with hypertonic 3.0 M KCl (0.8–3 MΩ resistance). In most experiments, however, the electrodes were filled with a nearly-isosmolar concentration of KCl (5–10 mOsm hypertonic with respect to the bathing solution; e.g., 135 mM KCl electrolyte for use with the modified Ringer's solution). These electrodes (0.5–2.0 MΩ resistance) were made from borosilicate omega-dot glass tubing, 1.5 mm o.d. by 1.17 mm i.d., pulled in two stages, and bevelled. In some cases it was necessary to allow several minutes for the oocyte-electrode seal to improve and reach a steady state, but afterwards the recording usually was stable for many hours and there was no evidence of swelling of the oocyte as with the hypertonic electrodes. Overall, in these and previous studies (not shown), oocyte whole cell resistances obtained after impalement with the larger isosmolar electrodes were similar to those obtained with the sharper electrodes filled with 3 M KCl (1.31 ± 0.06 MΩ, *n* = 188 compared to 1.30 ± 0.14 MΩ, *n* = 58 respectively).

Most receptor agonists, except for SCH 23390 and SCH 23388, were applied for 1 min. Under these conditions, in most oocytes 5-HT could be applied repeatedly at 30 min intervals without an apparent prolonged 'desensitization'. However, because SCH 23390 elicited a response only slowly, SCH 23390 and its enantiomer SCH 23388 were applied for 5 min. This longer exposure to SCH 23390 was followed by a prolonged 'desensitization' of the response to 5-HT, and therefore oocytes generally were discarded after exposure to SCH 23390 and other benzazepines that elicited an agonist-like response. On the other hand, if the oocyte did not respond to benzazepine (e.g., SCH 23388), there was no apparent 'desensitization' of the response to 5-HT.

Agonists produced an inward current response which was captured, stored, and analysed on a digital oscilloscope (Nicolet model 440, Nicolet Instrument Corp., Madison, Wisconsin, U.S.A.). Responses to 5-HT and other agonists applied for 60 s were quantified by measurement of the maximal or peak inward current and by integration of the response over a time base of 60 s from the initiation of the response. These integrals included the initial spike-like

response and the subsequent plateau when present, but not recovery from the plateau during washout of the agonist. Responses to SCH 23390 and SCH 23388 (applied for 5 min) were quantified by integration over a time base of 5 min.

The cell potential was also recorded. Current responses accompanied by a voltage loss of more than 2 mV during any part of the response were discarded. Typically the peak voltage loss was very brief and held to 1 mV or less.

Normalized responses In experiments with benzazepines, each oocyte was first exposed to 5-HT at 30-min intervals to confirm a stable response. Subsequently, the response to a benzazepine was obtained. Data show the ratio, computed for each oocyte, of the benzazepine response integral divided by the maximal 5-HT response integral. The purpose of this normalization was to correct for variability in the expression of 5-HT_{1C} receptors among oocytes.

Dose-response relationships Estimates of the EC₅₀ or IC₅₀ for various drug actions in oocytes were obtained by non-linear curve fitting (Marquardt-Levenberg algorithm; Sigma-Plot software, Jandel Scientific, Corte, Madera, California). Similar results were obtained by logit-log analysis when both types of analysis were used to estimate the EC₅₀ for 5-HT from the same set of data.

5-HT_{1C} receptor binding (choroid plexus)

Porcine choroid plexus was obtained from a local slaughterhouse and kept frozen. Membranes were prepared from the choroid plexus essentially as described by Pazos *et al.* (1985a, b) with minor modifications. The frozen choroid plexus was homogenized for 20 s in 10 volumes of 0.32 M sucrose at 4°C (Polytron homogenizer; Brinkman Instruments, Westbury, New York, U.S.A.), centrifuged at 6,000 *g* for 10 min at 4°C, and the membranes were then pelleted from that supernatant by centrifugation at 65,000 *g* for 15 min. The pellets were resuspended to the original volume in Tris-Ca buffer (50 mM Tris-Cl, pH 7.4, and 4 mM CaCl₂), and incubated at 37°C for 15 min. Finally, ascorbic acid (0.2% w/v) was added to the membrane suspension, and the preparation was stored at -70°C.

Ligand binding was measured by displacement of [¹²⁵I]-SCH 23982 in Tris-Ca buffer at 37°C for 60 min. Each tube contained 120 pM [¹²⁵I]-SCH 23982, non-labelled ligand ($\leq 10 \mu\text{M}$), and 100 μl (10 mg) of the choroid plexus membrane preparation in a total volume of 200 μl . Tissue containing bound ligands was separated by vacuum filtration and washed with cold Tris-Ca buffer. The radioactivity trapped on the glass-fibre filters was quantified (Beta-Plate; LKB, Gaithersburg, Maryland). Nonspecific binding was defined as that remaining in the presence of 10 μM SCH 23390. Binding affinities were estimated by computer analysis ('Accucomp', Lundon Software, Chagrin Falls, Ohio or 'Ligand' software kindly supplied by Dr Peter J. Munson, National Institutes of Health, Bethesda, Maryland, U.S.A.). Statistical comparisons were made by the Newman-Keuls procedure.

Materials

5-Hydroxytryptamine (5-HT) hydrochloride, 3-hydroxytryptamine (dopamine) hydrochloride, collagenase Type 1A, Leibovitz L-15 powder with L-glutamine, penicillin/streptomycin solution and sodium pyruvate were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). SCH 23390 ((R(+))-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) hydrochloride, SCH 23388 ((S(-))-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) hydrochloride, R(+)-SKF 38393 ((R(+))-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,

8-diol) hydrochloride), S(-)-SKF 38393, SKF 77434 (N-allyl-SKF 38393), SKF 82958 (6-chloro-N-allyl-SKF 38393), and spiperone were purchased from Research Biochemicals Incorporated (Natick, Massachusetts, U.S.A.). [¹²⁵I]-SCH 23982 ((+)-8-iodo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) was obtained from DuPont-NEN (Boston, Massachusetts, U.S.A.).

Results

Studies were conducted with 5-HT_{1C} mRNA synthesized from a cloned plasmid containing the complete 5-HT_{1C} receptor coding region. This receptor could be studied pharmacologically following injection of the clonal mRNA into oocytes. In non-injected oocytes or oocytes injected with a portion of the RNase-free water used to dissolve the mRNA, 5-HT and other compounds used in this study did not elicit inward current responses exceeding 10 nA (data not shown).

5-Hydroxytryptamine

The dose-response relationship for 5-HT in one oocyte that had been injected with clonal 5-HT_{1C} mRNA is shown in Figure 1a. The oocyte was exposed repeatedly to 5-HT at various concentrations. The EC₅₀ determined in this oocyte was 7.8 nM. The combined results from four additional similar experiments are shown in Figure 1b, indicating an average EC₅₀ of 13 nM for 5-HT. This is in good agreement with our measurement of the 5-HT binding to 5-HT_{1C} receptors in porcine choroid plexus, which had an apparent *K_i* of 19 nM (*pK_i* = 7.7 $\pm$ 0.1, mean $\pm$ s.e.mean, *n* = 3). These values also

Figure 1 Dose-response relationship for 5-hydroxytryptamine (5-HT) in oocytes injected with clonal 5-HT_{1C} mRNA. (a) Data are from one oocyte injected with 5-HT_{1C} mRNA. 5-HT at various concentrations was applied for 1 min at 30 min intervals, in the following sequence: 10 μM , 10 nM, 10 nM, 0.1 nM, 1 nM, 1 μM , 10 nM, 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 10 nM, 100 nM, and 1 μM . Each response (inward current) was integrated and the individual response integrals are plotted. The fitted curve indicates an EC₅₀ = 7.8 $\pm$ 1.1 nM (mean $\pm$ s.d.) in this oocyte. (b) Data are from four additional oocytes, each exposed repeatedly to 5-HT at various doses. Each response was integrated, and the integral was normalized to the integral of the response to 1 μM 5-HT measured in the same oocyte in order to account for variability in the expression of the injected 5-HT_{1C} mRNA. The responses to 1 μM 5-HT were -12.7 to -122 $\mu\text{A*s}$ (-45 $\pm$ 26 $\mu\text{A*s}$), and -1.78 to -9.15 μA (-3.9 $\pm$ 1.8 μA) at the peak of the response. The fitted curve indicates an EC₅₀ = 13 $\pm$ 17 nM (mean $\pm$ s.d.).

are in reasonably good agreement with other studies of 5-HT interaction with the 5-HT_{1C} receptor in mammalian cells (Hoyer *et al.*, 1985; Yagaloff & Hartig, 1985; Conn *et al.*, 1986; Peroutka, 1986; Hoyer *et al.*, 1989), although the 5-HT EC₅₀s determined in the choroid plexus (Conn *et al.*, 1986; Hoyer *et al.*, 1989) and in oocytes injected with brain RNA (Lübbert *et al.*, 1987b) are about 3–6 fold higher.

Methysergide (0.1 μ M) inhibited the response to 10 nM 5-HT by $90 \pm 3\%$ ($n = 3$). Spiperone had little or no effect at a concentration of 1.0 μ M ($4 \pm 9\%$ inhibition, $n = 4$), and at 10 μ M inhibited the response to 10 nM 5-HT by only $31 \pm 4\%$ ($n = 4$). Thus, methysergide was a potent antagonist while spiperone was a weak antagonist of the response to 5-HT in oocytes injected with clonal 5-HT_{1C} mRNA, similar to the 5-HT_{1C} receptor found in mammalian cells (Pazos *et al.*, 1985b; Yagaloff & Hartig, 1985; Conn *et al.*, 1986; Hoyer *et al.*, 1986b).

SCH 23390 and SCH 23388

SCH 23390 acted as an agonist in oocytes injected with clonal 5-HT_{1C} mRNA. However, the response to SCH 23390 developed much more slowly than the response to 5-HT, usually requiring exposure for 3 min or more rather than 1 min or less (Figure 2). Additionally, the response to SCH 23390 usually lacked the initial spike-like phase although many oscillations were present as is typical for this signal transduction system (Parker & Ivorra, 1990). Application of SCH 23390 (1 μ M) for 5 min did elicit a response in every oocyte tested, but that application also caused a prolonged reduction in the sensitivity to SCH 23390 and to 5-HT that lasted for several hours in some oocytes. Such 'desensitization' of similar responses in oocytes has been commonly observed and may be related to depletion of internal Ca²⁺ or other aspects of the signal transduction process (Berridge, 1989; Vasilets *et al.*, 1990). With 5-HT, we found that the 'desensitization' could be reduced by limiting the exposure to 1 min, allowing reproducible responses to be obtained at 30 min intervals.

Because of the need to apply SCH 23390 for several minutes and the ensuing hours-long desensitization, it was not generally practical to obtain reproducible responses to repeated application of SCH 23390 in individual oocytes. It was anticipated that there may be considerable variability among oocytes in the response to SCH 23390 due, at least in part, to variability in the expression of the 5-HT_{1C} receptors. To reduce such variability, the response to SCH 23390 (applied

Figure 2 Stereoselective agonist-like response to SCH 23390 in an oocyte injected with clonal 5-HT_{1C} mRNA. The oocyte was exposed to 30 nM 5-hydroxytryptamine (5-HT) for 1 min (a, one of 2 similar responses is shown), followed by 1 μ M SCH 23388 for 5 min (b, little or no response), followed by 1 μ M SCH 23390 for 5 min (c and d, each from the same response). The responses to SCH 23388 and SCH 23390 are shown at a higher gain than is the response to 5-HT. At (c), the response to SCH 23390 is shown on the same time scale as is the response to 5-HT above it. At (d), the response to SCH 23390 is shown on a longer time scale, identical to that for SCH 23388 above it. Calibration marks represent 200 nA and 60 s for each recording.

Figure 3 SCH 23390 dose-response relationship in oocytes injected with clonal 5-HT_{1C} mRNA. Oocytes ($n = 26$) were exposed at 30 min intervals repeatedly to 5-hydroxytryptamine (5-HT) (for 1 min) and then finally exposed once to one concentration of SCH 23390 for 5 min. To reduce variability among oocytes due to differences in the number or activity of expressed receptors, the response to SCH 23390 (integrated over 5 min) was normalized to the maximal response to 5-HT (integrated over 1 min) in each oocyte. The maximal responses to 5-HT were -2.72 to $-69.12 \mu\text{A}\cdot\text{s}$ ($-22.6 \pm 4.2 \mu\text{A}\cdot\text{s}$). The fitted curve indicates an EC₅₀ = 550 ± 240 nM (mean $\pm$ s.d.).

for 5 min) was normalized to the response to 5-HT (applied for 1 min) determined in each oocyte before the application of SCH 23390.

Figure 3 shows the dose-response relationship of the normalized integrals for SCH 23390, determined after one application of one dose of SCH 23390 per oocyte. The apparent EC₅₀ was 550 nM. The effect of SCH 23390 was clearly stereoselective because the enantiomer SCH 23388 (1 μ M) produced little or no response (Figures 2 and 5a). The stereoselectivity also was seen in the binding to 5-HT_{1C} receptors in porcine choroid plexus, where the apparent K_i for SCH 23390 was 6.3 nM ($pK_i = 8.20 \pm 0.08$, $n = 14$) compared to an apparent K_i for SCH 23388 of 1,100 nM ($pK_i = 5.96 \pm 0.2$, $n = 3$).

SKF 38393, SKF 77434 and SKF 82958

Other benzazepines were tested in a paradigm similar to that used for SCH 23390, except that 1-min applications were sufficient. As shown for SKF 38393 in Figure 4, at micromolar doses the dopamine D₁-selective agonists SKF 38393, SKF 77434, and SKF 82958 each elicited a response in oocytes injected with clonal 5-HT_{1C} mRNA. In individual oocytes, the responses to these benzazepines were smaller than the

Figure 4 Responses to SKF 38393 and 5-hydroxytryptamine (5-HT) in one oocyte injected with clonal 5-HT_{1C} mRNA. The oocyte was exposed to 1 μ M 5-HT for 1 min (a) followed by 100 μ M R(+)-SKF 38393 for 1 min (b). As in other oocytes, the responses to SKF 38393, SKF 77434, and SKF 82958 were similar in appearance but smaller than the response to 5-HT. The calibration marks represent 1 μ A and 60 s for each recording.

Figure 5 Benzazepine responses in oocytes injected with clonal 5-HT_{1C} mRNA. In each oocyte, the responses to the benzazepines were determined and normalized to the 5-hydroxytryptamine (5-HT) response in Figure 3, except that the SKF compounds were applied for 1 min and their responses were integrated over 1 min. The normalized responses were averaged among oocytes and are presented as mean $\pm$ s.e.mean. (a) Shown are normalized response to 1 μ M SCH 23390 (open column, 7 oocytes) and the lack of response to its enantiomer 1 μ M SCH 23388 (solid column, 7 oocytes). In 6 of these 7 oocytes there was no discernible response to 1 μ M SCH 23388. (b) Shown are the normalized responses to 100 μ M R(+)-SKF 38393 (open column, 5 oocytes), 100 μ M S(-)-SKF 38393 (solid column, 4 oocytes), 100 μ M ($\pm$)-SKF 77434 (hatched column, 4 oocytes) and 100 μ M ($\pm$)-SKF 82958 (cross-hatched column, 4 oocytes). There was no apparent stereoselectivity for R(+)-SKF 38393 (100 μ M), which is the more active enantiomer at the dopamine D₁-receptor.

* R(+)-SKF 38393 significantly different from ($\pm$)-SKF 77434 and ($\pm$)-SKF 82958, $P < 0.03$ (1-way ANOVA with *post-hoc* comparison using the method of contrasts).

response to 5-HT, but otherwise appeared similar to the response to 5-HT itself. The relative amplitudes of the responses to SKF 38393, SKF 77434 and SKF 82958 are shown in Figure 5b.

This effect of SKF 38393 was not stereoselective. The response to 100 μ M S(-)-SKF 38393 was as large as the response to 100 μ M R(+)-SKF 38393 (Figure 5b). Similarly, in the porcine choroid plexus the binding of SKF 38393 to 5-HT_{1C} receptors was not stereoselective; the apparent K_i for R(+)-SKF 38393 was 2.2 μ M ($pK_i = 5.67 \pm 0.1$, $n = 3$) while that for S(-)-SKF 38393 was 2.0 μ M ($pK_i = 5.70 \pm 0.06$, $n = 3$).

The responses to SKF 38393 in oocytes injected with 5-HT_{1C} mRNA were reasonably consistent with the binding parameters determined in choroid plexus. The response to 10 μ M R(+)-SKF 38393 appeared to be maximal, but only $29 \pm 10\%$ of the maximal response to 5-HT in the same oocytes ($n = 4$). Higher concentrations of R(+)-SKF 38393 did not elicit larger effects after normalization to the 5-HT response; the responses to 100 μ M and 1 mM SKF 38393 were $23 \pm 6\%$ ($n = 5$) and $27 \pm 8\%$ ($n = 5$) of the maximal response to 5-HT. The response to 1 μ M (+)-SKF 38393 was, on average, approximately half-maximal ($13 \pm 5\%$ of the response to 5-HT, $n = 5$). Furthermore, the binding of SKF 38393 in choroid plexus, like the response to SKF 38393 in oocytes injected with clonal 5-HT_{1C} RNA, was not stereoselective.

Derivatives of SKF 38393, ($\pm$)-SKF 77434 and ($\pm$)-SKF 82958 (100 μ M), also elicited 5-HT-like inward current responses in oocytes injected with 5-HT_{1C} mRNA although these responses were smaller than the responses to SKF 38393 after normalization to the 5-HT response (Figure 5b). Additionally, ($\pm$)-SKF 77434, and ($\pm$)-SKF 82958 bound to 5-HT_{1C} receptors in porcine choroid plexus with apparent K_i s of 4.1 μ M ($pK_i = 5.39 \pm 0.06$, $n = 3$) and 1.3 μ M ($pK_i = 5.89$

± 0.10 , $n = 3$) respectively. These micromolar K_i s should be considered estimates, since the highest ligand concentration used was 10 μ M. However, the data do indicate a good correspondence between the binding of these benzazepines to 5-HT_{1C} receptors in choroid plexus and their activation of the clonal 5-HT_{1C} receptor expressed in *Xenopus* oocytes.

Thus, it would appear that the stereoselective D₁-dopamine antagonist SCH 23390 is, at higher concentrations, a stereoselective 5-HT_{1C} agonist, while the stereoselective D₁-dopamine agonist is, at higher concentrations, a non-stereoselective 5-HT_{1C} agonist. Indeed, dopamine itself (100 μ M), like SKF 82958 and SKF 77434, also elicited a response in oocytes injected with 5-HT_{1C} RNA (not shown).

Discussion

In oocytes injected with clonal 5-HT_{1C} mRNA ('5-HT_{1C} oocytes'), SCH 23390 acted as a stereoselective agonist or partial agonist, and bound to choroid plexus 5-HT_{1C} receptors stereoselectively. Similarly, SCH 23390 and SKF 83566 have been found to be partial agonists (about 40% efficacy) in the choroid plexus, presumably by actions at the 5-HT_{1C} receptor (Hoyer *et al.*, 1989); stereoselectivity was not tested for this choroid plexus action. The response to SCH 23390 in 5-HT_{1C} oocytes, unlike the responses to the other benzazepines and 5-HT, activated slowly and often required several minutes of exposure to the compound. The rate of association of SCH 23390 with the dopamine D₁-receptor is relatively slow (Billard *et al.*, 1984; Andersen *et al.*, 1985; Niznik *et al.*, 1986). A similar slow association with the 5-HT_{1C} receptor could explain the need for longer application and the slow activation of the response observed in oocytes.

The EC₅₀ for SCH 23390 in 5-HT_{1C} oocytes was nearly two orders of magnitude greater than its binding K_i in porcine choroid plexus. This is consistent with other studies of SCH 23390 interaction with 5-HT_{1C} receptors and does not indicate an artefact of the cloned receptor expressed in oocytes. Hoyer *et al.* (1989) observed a similarly large difference between the SCH 23390 EC₅₀ (2.0 μ M) and binding K_i (5.5 nM) when both apparent 5-HT_{1C} receptor binding and functional activity (phosphatidylinositol hydrolysis) were measured in one tissue (choroid plexus). The reason for these EC₅₀/ K_i differences is not known, but slow association of SCH 23390 with the receptor and desensitization of the response might be contributing factors. In any case, the EC₅₀ for SCH 23390 estimated in oocytes injected with 5-HT_{1C} mRNA is reasonably close to the SCH 23390 EC₅₀ determined in choroid plexus, and in other comparisons, the actions of 5-HT and benzazepines in 5-HT_{1C} oocytes appeared to correlate well with the binding of these substances in porcine choroid plexus.

It is important to realize that as a partial agonist, SCH 23390 can act as an antagonist as well as an agonist at 5-HT_{1C} receptors (see Hoyer *et al.*, 1989). Indeed, in other experiments using oocytes injected with rat brain RNA, we have found that SCH 23390 can appear to be a stereoselective 5-HT_{1C} antagonist (data not shown). Thus, it should not be assumed that antagonism of a response by SCH 23390 necessarily distinguishes an action at D₁-dopamine versus 5-HT_{1C} receptors.

Three benzazepines regarded as selective D₁-dopamine agonists, SKF 38393, SKF 77434, and SKF 82958, at relatively high concentrations (1–100 μ M) also acted as agonists at the clonal 5-HT_{1C} receptor expressed in oocytes. Unlike the slow response to SCH 23390, these benzazepines elicited responses that were very similar in appearance to the response to 5-HT. The only apparent difference was that the responses to these SKF compounds were smaller in amplitude than the response to 5-HT recorded in the same oocytes. The responses to various concentrations of SKF 38393 were consistent with its binding K_i (2.0–2.2 μ M) to 5-HT_{1C} receptors in choroid plexus; the large difference between binding affinity and EC₅₀ noted with SCH 23390 was not apparent with SKF 38393.

However, SKF 77434 (100 μ M) and SKF 82958 (100 μ M) elicited responses that were smaller than those elicited by SKF 38393 (100 μ M). This was not explained by differences in the apparent binding affinity estimated in the choroid plexus. A likely explanation is that these benzazepines, at the 5-HT_{1C} receptor, are partial agonists with various degrees of efficacy. This hypothesis needs to be tested by measurement of complete dose-response curves.

Thus, at micromolar concentrations these benzazepines, which have been used as standard dopamine D₁-receptor agonists and antagonists, also act as 5-HT_{1C} agonists. Additionally, SCH 23390 has been found to interact with the related 5-HT₂ receptor (Hyttel, 1983; Hicks *et al.*, 1984; Ohlstein & Berkowitz, 1985; Hess *et al.*, 1986; Bischoff *et al.*, 1986; 1988; McQuade *et al.*, 1988; De Keyser *et al.*, 1989). Clearly, caution must be exercised in attributing all actions of SKF 38393, SKF 77434 and SKF 82958, particularly at micromolar concentrations, to activation of the dopamine D₁-receptor.

In oocytes injected with whole brain or striatal RNA, other investigators have reported responses to dopamine (10 μ M–1 mM) and to SKF 38393 (100 μ M) similar to the responses observed here in oocytes injected with clonal 5-HT_{1C} mRNA (Sumikawa *et al.*, 1984; Mahan *et al.*, 1990; Sakai *et al.*, 1990). In one report (Sumikawa *et al.*, 1984) it was found that 1 μ M methysergide blocked the response to 1 mM dopamine, as would be expected if dopamine acted through stimulation of a 5-HT_{1C} or 5-HT₂ receptor. Others, however, reported that neither mianserin nor ketanserin inhibited the response to dopamine or SKF 38393 (Mahan *et al.*, 1990; Sakai *et al.*, 1990), and it was suggested (Mahan *et al.*, 1990) that the responses may be due to activation of D₁-receptors thought to be coupled to phosphatidylinositol hydrolysis in mammalian cells (Undie & Friedman, 1990; Felder *et al.*, 1989a,b; but see Dyck, 1990; Rubinstein & Hitzemann, 1990).

References

- ANDERSEN, P.H., GRONVALD, F.C. & JANSEN, J.A. (1985). A comparison between dopamine-stimulated adenylate cyclase and ³H-SCH 23390 binding in rat striatum. *Life Sci.*, **37**, 1971–1983.
- BERRIDGE, M.J. (1989). Inositol 1,4,5-trisphosphate-induced calcium mobilization is localized in *Xenopus* oocytes. *Proc. R. Soc., B*, **238**, 235–243.
- BILLARD, W., RUPERTO, V., CROSBY, G., IORIO, L.C. & BARNETT, A. (1984). Characterization of the binding of ³H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci.*, **35**, 1885–1893.
- BISCHOFF, S., HEINRICH, M., KRAUSS, J., SILLS, M.A., WILLIAMS, M. & VASSOUT, A. (1988). Interaction of the D1 receptor antagonist SCH 23390 with the central 5-HT system: radioligand binding studies, measurements of biochemical parameters and effects on L-5-HTP syndrome. *J. Recept. Res.*, **8**, 107–120.
- BISCHOFF, S., HEINRICH, M., SONNTAG, J.M. & KRAUSS, J. (1986). The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT₂) receptors. *Eur. J. Pharmacol.*, **129**, 367–370.
- BRIGGS, C.A., POLLOCK, N.J., FRAIL, D.E., RAKOWSKI, R.F. & KEBABIAN, J.W. (1990). Benzazepine function at 5-HT_{1C} receptors expressed in *Xenopus* oocytes. *Soc. Neurosci. Abst.*, **16**, 1196.
- CONN, P.J. & SANDERS-BUSH, E. (1985). Serotonin-stimulated phosphoinositide turnover: Mediation by the S₂ binding site in rat cerebral cortex but not in subcortical regions. *J. Pharmacol. Exp. Ther.*, **234**, 195–203.
- CONN, P.J., SANDERS-BUSH, E., HOFFMAN, B.J. & HARTIG, P.R. (1986). A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4086–4088.
- DAWSON, T.M., BARONE, P., SIDHU, A., WAMSLEY, J.K. & CHASE, T.N. (1986). Quantitative autoradiographic localization of D-1 dopamine receptors in the rat brain: use of the iodinated ligand [¹²⁵I]SCH 23982. *Neurosci. Lett.*, **68**, 261–266.
- DE KEYSER, J., DIERCKX, R., VANDERHEYDEN, P., EBINGER, G. & VAUQUELIN, G. (1988). D1 dopamine receptors in human putamen, frontal cortex and calf retina: differences in guanine nucleotide regulation of agonist binding and adenylate cyclase stimulation. *Brain Res.*, **443**, 77–84.
- DE KEYSER, J., WALRAEVENS, H., CONVENTS, A., EBINGER, G. & VAUQUELIN, G. (1989). [³H]SCH 23390 labels a novel 5-hydroxytryptamine binding site in human blood platelet membranes. *Eur. J. Pharmacol.*, **162**, 437–445.
- DUMONT, J.N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.*, **136**, 153–179.
- DYCK, L.E. (1990). Effects of dopamine on phosphoinositide hydrolysis in slices of rat striatum and cortex. *Neurochem. Int.*, **17**, 77–82.
- FELDER, C.C., BLECHER, M. & JOSE, P.A. (1989a). Dopamine-1-mediated stimulation of phospholipase C activity in rat renal cortical membranes. *J. Biol. Chem.*, **264**, 8739–8745.
- FELDER, C.C., JOSE, P.A. & AXELROD, J. (1989b). The dopamine-1 agonist, SKF 82526, stimulates phospholipase-C activity independent of adenylate cyclase. *J. Pharmacol. Exp. Ther.*, **248**, 171–175.
- HARTIG, P.R. (1989). Molecular biology of 5-HT receptors. *Trends Pharmacol. Sci.*, **10**, 64–69.
- HESS, E.J., BATTAGLIA, G., NORMAN, A.B., IORIO, L.C. & CREESE, I. (1986). Guanine nucleotide regulation of agonist interactions at [³H]SCH 23390-labelled D₁ dopamine receptors in rat striatum. *Eur. J. Pharmacol.*, **121**, 31–38.
- HICKS, P.E., SCHOEMAKER, H. & LANGER, S.Z. (1984). 5HT-receptor antagonist properties of SCH 23390 in vascular smooth muscle and brain. *Eur. J. Pharmacol.*, **105**, 339–342.
- HOFFMAN, B.J. & MEZEY, E. (1989). Distribution of serotonin 5-HT_{1C} receptor mRNA in adult rat brain. *FEBS Lett.*, **247**, 453–462.
- HOYER, D. (1988). Molecular pharmacology and biology of 5-HT_{1C} receptors. *Trends Pharmacol. Sci.*, **9**, 89–94.
- HOYER, D. & KARPF, A. (1988). [¹²⁵I]SCH 23982, a 'selective' D-1 receptor antagonist, labels with high affinity 5-HT_{1C} sites in pig choroid plexus. *Eur. J. Pharmacol.*, **150**, 181–184.
- HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT₁ and 5-HT₂ recognition sites in rat and pig brain membranes: Radioligand binding studies with [³H]5-HT, [³H]8-OH-DPAT, (–)[¹²⁵I]iodocyanopindolol, [³H]mesulergine and [³H]ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.
- HOYER, D., PAZOS, A., PROBST, A. & PALACIOS, J.M. (1986a). Seroton-

- in receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT₂ recognition sites. *Brain Res.*, **376**, 97–107.
- HOYER, D., SRIVATSA, S., PAZOS, A., ENGEL, G. & PALACIOS, J.M. (1986b). [¹²⁵I]LSD labels 5-HT_{1C} recognition sites in pig choroid plexus membranes, comparison with [³H]mesulergine and [³H]5-HT binding. *Neurosci. Lett.*, **69**, 269–274.
- HOYER, D., WAEBER, C., SCHOEFFTER, P., PALACIOS, J.M. & DRAVID, A. (1989). 5-HT_{1C} receptor-mediated stimulation of inositol phosphate production in pig choroid plexus. A pharmacological characterization. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **339**, 252–258.
- HYTTTEL, J. (1983). SCH 23390—the first selective dopamine D-1 antagonist. *Eur. J. Pharmacol.*, **91**, 153–154.
- JENCK, F., BROEKKAMP, C.L.E. & VAN DELFT, M.L. (1990). 5-HT_{1C} receptors in the serotonergic control of periaqueductal gray induced aversion in rats. *Psychopharmacol.*, **100**, 372–376.
- JULIUS, D., MACDERMOTT, A.B., AXEL, R. & JESSELL, T.M. (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science*, **241**, 558–564.
- LAHOSTE, G.J. & MARSHALL, J.F. (1990). Nigral D₁ and striatal D₂ receptors mediate the behavioural effects of dopamine agonists. *Behav. Brain Res.*, **38**, 233–242.
- LESTER, H.A. (1988). Heterologous expression of excitability proteins: route to more specific drugs? *Science*, **241**, 1057–1063.
- LÜBBERT, H., HOFFMAN, B.J., SNUTCH, T.P., VAN DYKE, T., LEVINE, A.J., HARTIG, P.R., LESTER, H.A. & DAVIDSON, N. (1987). cDNA cloning of a serotonin 5-HT_{1C} receptor by electrophysiological assays of mRNA-injected *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 4332–4336.
- LÜBBERT, H., SNUTCH, T.P., DASCAL, N., LESTER, H.A. & DAVIDSON, N. (1987b). Rat brain 5-HT_{1C} receptors are encoded by a 5–6 kb mRNA size class and are functionally expressed in injected *Xenopus* oocytes. *J. Neurosci.*, **7**, 1159–1165.
- MAHAN, L.C., BURCH, R.M., MONSMA, F.J., JR. & SIBLEY, D.R. (1990). Expression of striatal D₁ dopamine receptors coupled to inositol phosphate production and Ca²⁺ mobilization in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 2196–2200.
- MCQUADE, R.D., FORD, D., DUFFY, R.A., CHIPKIN, R.E., IORIO, L.C. & BARNETT, A. (1988). Serotonergic component of SCH 23390: *in vitro* and *in vivo* binding analyses. *Life Sci.*, **43**, 1861–1869.
- MENGOD, G., NGUYEN, H., LE, H., WAEBER, C., LÜBBERT, H. & PALACIOS, J.M. (1990). The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by *in situ* hybridization histochemistry, comparison with receptor binding distribution. *Neurosci.*, **35**, 577–591.
- MOLINEAUX, S.M., JESSELL, T.M., AXEL, R. & JULIUS, D. (1989). 5-HT_{1C} receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci., U.S.A.*, **86**, 6793–6797.
- MOLLOY, A.G. & WADDINGTON, J.L. (1987). Assessment of grooming and other behavioural responses to the D-1 dopamine receptor agonist SK & F 38393 and its R- and S-enantiomers in the intact adult rat. *Psychopharmacol.*, **92**, 164–168.
- NICKLAUS, K.J., MCGONIGLE, P. & MOLINOFF, P.B. (1988). [³H]SCH 23390 labels both dopamine-1 and 5-hydroxytryptamine_{1C} receptors in the choroid plexus. *J. Pharmacol. Exp. Ther.*, **247**, 343–348.
- NIZNIK, H.B., OTSUKA, N.Y., DUMBRILLE-ROSS, A., GRIGORIADIS, D., TIRPAK, A. & SEEMAN, P. (1986). Dopamine D₁ receptors characterized with [³H]SCH 23390: Solubilization of a guanine nucleotide-sensitive form of the receptor. *J. Biol. Chem.*, **261**, 8397–8406.
- OHLSTEIN, E.H. & BERKOWITZ, B.A. (1985). SCH 23390 and SK&F 83566 are antagonists at vascular dopamine and serotonin receptors. *Eur. J. Pharmacol.*, **108**, 205–208.
- PARKER, I. & IVORRA, I. (1990). Localized all-or-none calcium liberation by inositol trisphosphate. *Science*, **250**, 977–979.
- PAZOS, A., HOYER, D. & PALACIOS, J.M. (1985a). Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: Evidence for species differences in brain serotonin-2 receptors. *Eur. J. Pharmacol.*, **106**, 531–538.
- PAZOS, A., HOYER, D. & PALACIOS, J.M. (1985b). The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.*, **106**, 539–546.
- PEROUTKA, S.J. (1986). Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. *J. Neurochem.*, **47**, 529–540.
- PEROUTKA, S.J. (1988). 5-Hydroxytryptamine receptor subtypes: molecular, biochemical and physiological characterization. *Trends Neurosci.*, **11**, 496–500.
- RUBINSTEIN, J.E. & HITZEMANN, R.J. (1990). Further evidence against the coupling of dopamine receptors to phosphoinositide hydrolysis in rat striatum. *Biochem. Pharmacol.*, **39**, 1965–1970.
- SAKAI, Y., SEKIGUCHI, M., OKAMOTO, K., KUWANO, R. & TAKAHASHI, Y. (1990). Dopamine receptors expressed in the *Xenopus* oocytes injected with bovine striatal RNA. *Mol. Brain Res.*, **7**, 183–187.
- SCHMIDT, A.W. & PEROUTKA, S.J. (1989). 5-Hydroxytryptamine receptor “families”. *FASEB J.*, **3**, 2242–2249.
- SIGEL, E. (1990). Use of *Xenopus* oocytes for the functional expression of plasma membrane proteins. *J. Memb. Biol.*, **117**, 201–221.
- SKARSFELDT, T. & LARSEN, J.J. (1988). SCH 23390—a selective dopamine D-1 receptor antagonist with putative 5-HT₁ receptor agonistic activity. *Eur. J. Pharmacol.*, **148**, 389–395.
- SMITH, A.A., BROOKER, T. & BROOKER, G. (1987). Express of rat mRNA coding for hormone-stimulated adenylate cyclase in *Xenopus* oocytes. *FASEB J.*, **1**, 380–387.
- SNUTCH, T.P. (1988). The use of *Xenopus* oocytes to probe synaptic communication. *Trends Neurosci.*, **11**, 250–256.
- SUMIKAWA, K., PARKER, I. & MILEDI, R. (1984). Messenger RNA from rat brain induces noradrenaline and dopamine receptors in *Xenopus* oocytes. *Proc. R. Soc., B*, **223**, 255–260.
- SUMIKAWA, K., PARKER, I. & MILEDI, R. (1986). *Xenopus* oocytes as a tool for molecular cloning of the genes coding for neurotransmitter receptors and voltage-operated channels. *Fortschritte Zoologie*, **33**, 127–139.
- UNDIE, A.S. & FRIEDMAN, E. (1990). Stimulation of a dopamine D₁ receptor enhances inositol phosphates formation in rat brain. *J. Pharmacol. Exp. Ther.*, **253**, 987–992.
- VASILETS, L.A., SCHMALZING, G., MÄDEFESSEL, K., HAASE, W. & SCHWARZ, W. (1990). Activation of protein kinase C by phorbol ester induces downregulation of the Na⁺/K⁺-ATPase in oocytes of *Xenopus laevis*. *J. Memb. Biol.*, **118**, 131–142.
- YAGALOFF, K.A. & HARTIG, P.R. (1985). ¹²⁵I-Lysergic acid diethylamide binds to a novel serotonergic site on rat choroid plexus epithelial cells. *J. Neurosci.*, **5**, 3178–3183.
- YAMAMOTO, T. & KEBABIAN, J.W. (1989). [¹²⁵I]SCH23982 binds to a serotonin receptor (and not to a D-1 dopamine receptor) in the rat choroid plexus. *Biogen. Amines*, **6**, 241–246.

(Received April 3, 1991)

Revised August 5, 1991

Accepted August 22, 1991)