NEUROCHEMICAL PROFILE OF ELTOPRAZINE

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CONTENTS

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SUMMARY

In this paper we present the neurochemical profile of eltoprazine, a drug that specifically inhibits offensive aggression. Eltoprazine interacts selectively with serotonin (5-HT) receptor subtypes (K_i-values for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptors are 40, 52 and 81 nM respectively). Affinity for other neurotransmitter receptors is much lower (K_i-values > 400 nM) than for 5-HT_1 receptors. The selective interaction with 5-HT_1 receptor subtypes is confirmed by *in vitro* autoradiographic studies using radiolabelled eltoprazine. The overall distribution of [^{3}H]eltoprazine bears a strong resemblance to the localization of 5-HT_1 binding sites labelled by [^{3}H]5-HT, although some differences are observed. Eltoprazine (1μM) inhibits the forskolin stimulated c-AMP production in hippocampus slices of the rat, indicating an agonistic action on the 5-HT_{1A} receptor. The K^+ stimulated release of 5-HT from rat cortex slices is inhibited by eltoprazine (pD_2 = 7.8). The maximal response, however, was clearly less than that of the full agonist 5-HT, indicating partial agonistic activity on the 5-HT_{1B} receptor (α = 0.5). Eltoprazine has a weak antagonistic action (IC_{50} = 7μM) on the 5-HT_{1C} receptor as revealed by inhibition of the 5-HT-induced accumulation of inositol phosphates in the choroid plexus of the pig. *In vivo*, eltoprazine reduces 5-HIAA levels in the striatum, without affecting the 5-HT levels. Eltoprazine also reduces the 5-HT synthesis rate as shown by 5-HTP accumulation after decarboxylase inhibition. These data indicate that eltoprazine acts as a 5-HT agonist *in vivo* in a dose range that affects aggressive behaviour (0.3-3 mg/kg p.o.).

Taken together from a variety of neurochemical studies there is strong evidence both *in vitro* and *in vivo* that the pharmacological actions of eltoprazine can be attributed to an interaction with the 5-HT system, most probably via a (partial) agonistic action on 5-HT_{1A} and 5-HT_{1B} receptors.

1. INTRODUCTION

Eltoprazine (DU 28853) is the major representative of a class of compounds labelled "serenics" due to their specific inhibition of the offensive components of agonistic behaviour, without causing sedation, muscle relaxation or inhibition of defensive or social behaviours, in a number of animal models /1-3/. The mechanism of action of eltoprazine is not clear, but behavioural and

neurochemical investigations point towards an interaction with central serotonergic (5-HT) systems.

Over the last two decades it has become clear that serotonin plays an important role in the regulation of agonistic and other behaviours (for reviews /4,5/). Early workers hypothesized that central serotonergic systems exert an inhibitory influence over aggression /6/. However, matters seem to be more complex, as different forms of aggressive behaviour can be distinguished according to the underlying motivations, postures displayed and neuronal substrates engaged /2,7-11/.

It is conceivable that the involvement of serotonin in any type of aggressive behaviour is not only dependent on the differential anatomical distribution of serotonergic fibers /12/, but also on the participation of one or more serotonergic receptor subtypes that display specific pharmacological (e.g. /13/) and anatomical /14/ characteristics.

At present, three main 5-HT receptor subclasses have been defined: 5-HT_1, 5-HT_2 and 5-HT_3 /15/. Radioligand binding studies have revealed at least four subtypes of the 5-HT_1 binding site (5-$HT_{1A,1B,1C \text{ and } 1D}$) /16,17/. The demonstration of the coupling of 5-HT binding sites to specific transducing systems provides direct evidence that these sites are functional receptors. For instance, the 5-HT_{1A} receptor is coupled to adenylate cyclase both in a positive way /18,19/, as well as in a negative way /20,21/. The 5-HT_{1B} and 5-HT_{1D} binding sites are only negatively coupled to adenylate cyclase /22,23/. The 5-HT_{1C} receptor subtype is not linked to adenylate cyclase, but positively coupled to the phosphoinositide pathway via phospholipase C /24,25/. With respect to the other 5-HT receptors it is known that the 5-HT_2 receptor is linked to the phosphoinositide pathway /26/, while the 5-HT_3 receptor is directly coupled to an ion channel /27/. The 5-HT receptor subtypes have not only been functionally linked to second messenger systems, but also to specific physiological processes. A well known example is the modulation of 5-HT release via autoreceptors located on the 5-HT terminals. Stimulation of the 5-HT autoreceptor results in inhibition of 5-HT release /28,29/. In the rat, this autoreceptor has been characterized as a 5-HT_{1B} receptor /30-32/. In other species, such as pig /33/ and guinea pig /34,35/, it has been suggested that this receptor is more related to the 5-HT_{1D} binding site. Given that specific 5-HT receptors are linked to specific biochemical and physiological processes, this can be used to monitor the functional activity of a given compound on these receptor subtypes.

To gain insight into the mechanism of action of eltoprazine, we have studied its neurochemical profile in rat brain. In this paper we

present the affinity of eltoprazine for 32 different receptors, including seven 5-HT subtypes. In addition we present the characteristics and neuroanatomical distribution of [^{3}H]eltoprazine binding in rat brain as studied by *in vitro* receptor autoradiography. The agonistic or antagonistic properties of eltoprazine on various 5-HT receptor subtypes are described in functional studies on second messenger responses and 5-HT neurotransmission both *in vitro* and *in vivo*.

II. MATERIALS AND METHODS

2.1 Membrane binding studies

All binding assays were performed according to well-documented methods summarized in Table 1.

Drug solutions were pipetted manually in all displacement experiments, whilst the [^{3}H]ligand solutions and tissue suspensions were pipetted automatically by a Filterprep 101 (Ismatec, Zürich, Switzerland), which further performed the assays up to and including the addition of Scintillation Emulsifier-299 (Packard) to the glass fiber filters (Watman GF/B), collected in plastic minivials (Packard). Overnight equilibration was followed by counting of the content of the vials for tritium in a Liquid Scintillation Counter (Packard B460). Concentrations of unlabelled drug causing 50% displacement of the specific binding of a [^{3}H]label (IC_{50} values) were obtained by computerized log-probit linear regression analysis of data obtained in experiments in which four to six different concentrations of the test compound were used. Inhibition constant (K_i) values were calculated using the Cheng-Prusoff equation: $K_i = IC_{50}/(1+S/K_d)$ in which S represents the concentration of the [^{3}H]label. Average K_i-values were calculated from at least three values obtained from independent experiments, that is, in experiments performed on different days with different membrane preparations. All incubations were done in triplicate.

2.2 In vitro autoradiography

Tissue preparation and autoradiographic procedures were performed according to previously published methodology /64/. In brief, male Wistar rats were killed by decapitation, and brains were rapidly frozen on powdered dry ice. Coronal brain sections (20 μm), mounted onto gelatin coated microscope slides, were preincubated for 30 min in 0.17 M Tris-HCl (pH 7.6) and 4 mM

TABLE 1: Receptor binding methodology

Receptor	Tissue	[^{3}H]-Ligand	Reference
α_1-adrenergic	total brain	PRAZOSINE	/36/
α_2-adrenergic	tot.br.-cereb.	CLONIDINE	/37/
$\beta_{1\,2}$-adrenergic	cerebral cortex	DHA[1]	/38/
dopamine-D_1	n. caudate	DOPAMINE	/39/
dopamine-D_2	c. striatum	SPIPERONE	/40/
5-HT_{1A}	frontal cortex	8-OH-DPAT	/41/
5-HT_{1B}	frontal cortex	SEROTONIN[2]	/42/
5-HT_{1C}	choroid plexus[3]	SEROTONIN	/43/
5-HT_{1D}	n. caudate[4]	SEROTONIN[5]	/17/
5-HT_2	frontal cortex	SPIPERONE	/44/
5-HT_3	nb-g cells[6]	GR 38032F	/45/[7]
5-HT_{uptake}	frontal cortex	PAROXETINE	/46/
tryptamine	cerebral cortex	TRYPTAMINE	/47/
histamine-H_1	total brain	MEPYRAMINE	/48/
muscarine-M_1	hippocampus	PIRENZEPINE	/49/
muscarine-M_2	atrium (heart)	N-Me-SCOPOLAMINE	/49/
muscarine-M_3	submand. gland	N-Me-SCOPOLAMINE	/49/
μ-opiate	tot. br.-cereb.	NALOXONE	/50/
κ-opiate	tot.br.-cereb.	EKC	/51/
δ-opiate	tot.br.-cereb.	DADLE	/52/
$Ca^{++}{}_{chan}$-(DHP)	cerebral cortex	NITRENDIPINE	/53/
$Ca^{++}{}_{chan}$-(VER)	forebrain	D-888	/54/
benzodiazepine	total brain	DIAZEPAM	/55/
$GABA_A$	cerebellum	DH-MUSCIMOL	/56/
glycine	medulla + pons	STRYCHNINE	/57/
NMDA	tot.br.-cereb.	CGS 19755	/58/
LTD_4	lung[8]	LTD_4	/59/
SIGMA	total brain	(+)-3-PPP	/60/
TRH	tot.br.-cereb.	MeTRH	/61/
CCK (CNS)	cerebral cortex	CCK-8	/62/
CCK (pancreas)	pancreas	CCK-8	/62/
Substance P	t.br.-cer.-cortex	SUBSTANCE P	/63/

[1]: DHA = dihydroalprenolol. In this assay 10^{-6} M serotonin is added to prevent binding of [^{3}H]-DHA to 5-HT_1 receptors. [2/5]: In these assays 3.10^{-8}M unlabelled 8-OH-DPAT and 3.10^{-8}M DOI are added to the incubation medium in order to block 5-HT_{1A} and 5-HT_{1C} receptors respectively. [3]: Pig. [4]: Bovine. [6]: Cultivated mouse neuroblastoma-glioma cells. [7]: Method identical to this reference, except [^{3}H]GR 38032F was used as ligand, instead of [^{3}H]-ICS 205,930. [8]: guinea pig.

$CaCl_2$. After drying, the sections were incubated for 60 min at room temperature with either 12 nM [^{3}H]eltoprazine (Amersham; 23-31 Ci/mmol) in 400 μl 0.17 M Tris-HCl (pH 7.6), 4 mM $CaCl_2$ and 0.01% ascorbic acid, or 1.8 nM [^{3}H]5-HT (NEN; 20 Ci/mmol) in the same incubation buffer supplemented with 10 μm pargyline and 1 μm zimeldine (in order to block degradation via MAO and 5-HT reuptake, respectively). Nonspecific binding was defined in the presence of either 1 μM 5-HT or 1 μM eltoprazine. In equilibrium saturation experiments, consecutive sections from 6 rat brains were incubated with increasing concentrations of [^{3}H]eltoprazine (5-40 nM) or [^{3}H]5-HT (1-10 nM). After the incubation, sections were rinsed twice for 3 min at 4°C in fresh preincubation buffer followed by a short dip in ice-cold distilled water. Slides were dried at 60°C and opposed to tritium-sensitive Amersham Ultrofilm together with ^{3}H-Standard microscales (Amersham RPA.506 and RPA.507) for a period of 7 weeks. Following standard developing and fixing procedures the autoradiograms were analysed with a Vidas image analysis system (Kontron, Munich, FRG). The optical density measurements were converted to fmol of ligand bound/mg tissue equivalent using the ^{3}H-microscales from the same film. The neuroanatomical regions were defined with use of the stereotaxic brain atlas of Paxinos and Watson /65/. Specific binding values were obtained by subtracting nonspecific binding from total binding. The saturation data were analysed with the nonlinear curvefitting program LIGAND /66/, and are presented as means ± S.E.M. of average measurements from 6 rats.

2.3 **Adenylate cyclase activity**

Adenylate cyclase activity was measured in hippocampal tissue. The assay conditions were slightly modified from those published by Devivo and Maayani /20/. Hippocampi were homogenized in 9 volumes of Tris-HCl (pH 7.4) containing 300 mM sucrose, 1 mM EGTA, 5 mM EDTA and 5 mM dithiothreitol. The homogenate was centrifuged at 500 x g for 5 min. The supernatant was then recentrifuged at 35000 x g for 10 min. The pellet was then resuspended in the same volume as before and diluted to a concentration of 0.3 mg protein/ml. Fifty microliters of this membrane suspension were finally added to 200 μl of the assay medium. The final components and their concentrations were: 10 μM forskolin, 100 mM NaCl, 10 μM GTP, 2mM $MgCl_2$, 0.2 mM ATP; 1 mM cyclic AMP, 80 mM Tris-HCl, 60 mM sucrose, 0.2 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 1 μCi [α-^{32}P]ATP, 4 mM theophylline, 10 μg creatinine phosphokinase, 5 mM

creatinine phosphate and various concentrations of test compounds. The assay medium was preincubated for 5 min at 30°C before addition of the membrane suspension. The incubation was 5 min at 30°C and the reaction was stopped by addition of 100 μl of a solution containing 2% sodium lauryl sulphate, 40 mM ATP and 1.4 mM c-AMP at pH 7.5

The [α-^{32}P]c-AMP produced by the homogenate was separated from the substrate [α-^{32}P]ATP as described by Salomon et al. /67/ firstly on Dowex AG50W-X4 columns followed by further separation on neutral alumina columns. The recovery of this column procedure was 70-80%. Effects of test compounds were expressed as percentages of the forskolin stimulated c-AMP production. Statistical analyses were performed with the two-tailed Student t-test. A probability of $P < 0.05$ was regarded as significant.

2.4 **Release measurements**

Parietal cortex tissue of male Wistar rats (180 - 220 g) was obtained after decapitation of the animals. Immediately after removal, the tissue was placed in ice-cold Krebs-Ringer bicarbonate (KRB) medium. After dissection, the pieces of tissue were minced by passing them twice through a McIlwain tissue chopper set at 225 μm. Prior to the second pass the surface was rotated 90 degrees. The resulting slices measured 2 x 0.2 x 0.2 mm and were collected in ice-cold KRB medium, containing 121 mM NaCl, 1.87 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 1.22 mM $CaCl_2$, 25 mM $NaHCO_3$ and 10 mM D(+)-glucose. The medium was kept under a constant atmosphere of 95% 0_2 and 5% CO_2.

Tissue slices were washed twice in 5 ml of fresh medium and then incubated in a shaking waterbath for 10 min in 10 ml KRB medium at 37°C under a constant flow of 95% O_2 and 5% CO_2. Then the slices were incubated for 15 min with 0.1 μM [^{3}H]5-HT (NEN, 20 Ci/mmol). At the end of this incubation period, the tissue slices were washed twice with medium (2 ml) and aliquots of approximately 10 mg (wet weight) were transferred to each of the 24 chambers of a superfusion apparatus (chamber volume: 0.2 ml).

Subsequently, the slices were superfused with KRB medium at a rate of 0.25 ml/min at 37°C. The 5-HT uptake blocker, fluvoxamine (10 μM) was present throughout the superfusion to prevent the reuptake of 5-HT into the nerve terminals. After superfusion of 45 min, nine successive 15 min fractions of the superfusate were collected (from t=45 min to t=180). Stimulation of the release was evoked by exposing the tissue for 5 min to KRB medium with an

elevated K^+ concentration (25 mM) at t=60, 105 and 150 representing respectively S_1, S_2 and S_3. Agonists and antagonists were added to the KRB medium 15 and 20 min respectively before stimulation. At the end of the superfusion experiments the remaining radioactivity was extracted from the tissue with 4 ml 0.1N HCl.

The radioactivity in the collected fractions was determined by liquid scintillation counting. Tritium efflux was calculated as the fraction of the total amount of tritium present at the onset of the collection period (fractional rate of tritium efflux). The total amount of radioactivity released in the stimulated fractions was corrected for basal release (assumed to decline linearly). In order to quantify drug induced changes of stimulated release, the ratio of the overflow evoked by S_3, S_2 and S_1 (S_3/S_1 and S_2/S_1) was determined and expressed as a percentage of control.

In all experiments data were collected in triplicate. The data are expressed as means ± SEM of n independent experiments. Two-tailed Student's t-test was used for comparison of mean values. Significance was accepted when $p < 0.05$. The drug effects, as derived from the concentration response curves, are expressed in terms of intrinsic activity (α) and apparent affinity of the agonists (pD_2) and the antagonists (pA_2).

2.5 **Phosphatidyl inositol turnover measurements**

Pig choroid plexi were freshly obtained from a local slaughterhouse. The tissue was minced using a McIlwain tissue chopper. Slices of pig choroid plexus were preincubated for 30 min in KRB buffer at 37°C under 95% O_2 + 5% CO_2 atmosphere. Aliquots (20μl) of the slices suspension were transferred to tubes containing 300 μl buffer with 5 mM lithium chloride and ^{3}H-myoinositol (1μCi/tube). After 30 min, 40 μl of a solution containing an antagonist was added and the incubation was continued for 15 min. Then 40 μl of a solution containing the agonist (e.g. serotonin) was added and the incubation was continued for another 60 min.

Then the incubation was stopped by vortexing with 1 ml chloroform/methanol (1:2; v/v) and another 300 μl chloroform followed by 300 μl water was added. All tubes were vortexed after both additions. After centrifugation (10 min, 1000 g, 20°C), 700 μl of the water phase was used for determination of the different inositol phosphates by anion exchange chromatography as described by Berridge et al. /68/. Liquid scintillation counting of 100 μl of the organic phase was used to determine incorporation of inositol in the lipids. Radioactivity in the phosphate fraction was

divided by that in the lipid fraction and expressed as a percentage of control values.

2.6 Determination of monoamines and metabolites

Male Wistar rats (200 - 300 g) were treated orally in groups of five animals, one hour before decapitation. The brain tissue (striatum) was dissected out and frozen rapidly on dry ice. The frozen brain tissue was weighed and homogenised in 10 volumes (w/v) of ice-cold 0.1 M $HClO_4$/0.05 M phosphate buffer (pH 3) containing N-methyl-serotonin as an internal standard. Samples were then centrifuged at 8800 g for 1.5 minutes using a microfuge (Beckman). Aliquots (75μl) of the clear supernatant were analysed using a HPLC system (Hewlett Packard 1085) with a reversed phase column (Zorbax-C8, 15 x 0.46 cm, particle size 7.5μm). The mobile phase was 0.05 M ammonium phosphate/1.5% n-propanol (pH 3) containing 2.55 mM sodium octylsulphonate, 0.1 mM EDTA, 0.1 M $NaClO_4$ and 0.5 mM triethylamine. A constant flow of 2 ml/min was delivered, while the column temperature was maintained at 18 ± 0.5°C. DA, HVA, DOPAC, 5-HT and 5-HIAA were detected electrochemically with a BAS model LC-4A from Bioanalytic Systems at 750 mV with a glassy carbon electrode versus an Ag/AgCl reference electrode. The controller was set at 10 nA full scale and the output was recorded at a Hewlett Packard 3392 integrator.

Calculations were made with the internal standard method using the peak height value /69/.

2.7 5-HT turnover measurements

Turnover measurements were done by measuring the accumulation of 5-HTP after inhibition of the aromatic amino acid decarboxylase by NSD 1015. Male Wistar rats (200-300g) were treated with test compounds and after 30 min with 100 mg/kg i.p. NSD 1015. Then, after 30 min, rats were killed and the brain tissues were dissected and frozen on dry ice. The levels of 5-HTP were determined using the HPLC procedure as described above.

2.8 Drugs

The drugs used were: 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) (Research Biochemical Inc., USA); fluvoxamine, flesinoxan, eltoprazine and TFMPP (1-(3-trifluoromethylphenyl) piperazine hydrochloride) (Duphar, The Netherlands); mianserine

(Organon, The Netherlands); spiperone (Janssen, Belgium); 5-hydroxytryptamine creatine sulphate and pargyline (Sigma, USA).

All radioactively labelled compounds used in this study were obtained either from New England Nuclear or Amersham. Both [^{3}H]eltoprazine and [^{3}H]GR38032 were custon -synthesized by Amersham.

III. RESULTS

3.1 Receptor-binding profile

From its binding profile it is evident that eltoprazine's highest affinity is for 5-HT_1 receptors (see Table 2) and it is equipotent on 5-HT_{1A} and 5-HT_{1B}, but somewhat less on 5-HT_{1C} with much lower potency on 5-HT_{1D} receptors.

With respect to other receptors, however, there is a difference by at least a factor of 10 between the affinity for 5-HT_1 and β_{12}- and α_1-adrenergic receptors, the receptors for which eltoprazine has the next highest affinity.

The binding profile of eltoprazine, together with the direct binding data obtained with [^{3}H]eltoprazine, clearly demonstrates the compound to be a selective 5-HT_1 ligand (selective with respect to all receptors other than 5-HT_1). As far as receptor binding affinities are concerned, eltoprazine closely resembles serotonin (Table 3) except for the relatively low affinity for the 5-HT_{1D} receptor.

Furthermore, the serotonergic binding profiles of other compounds used in this study are given in Table 3.

8-OH-DPAT and flesinoxan display high selectivity towards 5-HT_{1A} receptors, whereas TFMPP shows moderate selectivity for 5-HT_{1C} receptors. Spiperone shows high selectivity for the 5-HT_2 receptors, whereas mianserine shows high affinity for both the 5-HT_2 and the 5-HT_{1C} receptor. Fluvoxamine was included because of its selective 5-HT uptake inhibiting properties.

3.2 Distribution of [^{3}H]eltoprazine and [^{3}H]5-HT binding sites in rat brain sections

In vitro autoradiographic studies with [^{3}H]eltoprazine demonstrate a widespread but heterogenous distribution throughout the rat brain (Fig. 1). Nearly all the binding is to grey matter areas with non-significant levels present in white matter tracts such as the corpus callosum. The nonspecific binding,

TABLE 2: Receptor binding profile of eltoprazine

Receptor	K_i(nM)	± S.E.M.	(n = x)
α_1-adrenergic	790	± 110	(3)
α_2-adrenergic	3,100	± 500	(3)
β_{12}-adrenergic	420	± 110	(3)
dopamine-D_1	>10,000		(2)
dopamine-D_2	1,100	± 90	(3)
5-HT_{1A}	40	± 5	(5)
5-HT_{1B}	52	± 12	(3)
5-HT_{1C}	81	± 2	(3)
5-HT_{1D}	390	± 30	(3)
5-HT_2	1,700	± 200	(3)
5-HT_3	1,300	± 400	(3)
5-HT_{uptake}	>10,000		(2)
tryptamine	77,000	± 6,000	(3)
histamine-H_1	3,900	± 1,300	(3)
muscarine-M_1	>10,000		(2)
muscarine-M_2	>10,000		(2)
muscarine-M_3	>10,000		(2)
μ-opiate	>10,000		(2)
κ-opiate	>10,000		(2)
δ-opiate	>10,000		(2)
Ca^{++}-(DHP)	>10,000		(2)
Ca^{++}_{chan}-(VER)	>10,000		(2)
benzodiazepine	>10,000		(2)
$GABA_A$	>10,000		(2)
glycine	>10,000		(2)
NMDA	>10,000		(2)
TRH	>10,000		(2)
LTD_4	>10,000		(2)
SIGMA	9,300	± 800	(3)
CCK(CNS)	>10,000		(2)
CCK (pancreas)	>10,000		(2)
Substance P	>10,000		(2)

TABLE 3

Serotonergic binding profiles

Compound: Receptor	8-OH-DPAT K_i(nM)	flesinoxan K_i(nM)	TFMPP K_i(nM)	5-HT K_i(nM)	fluvoxamine K_i(nM)	mianserin K_i(nM)	spiperone K_i(nM)
5-HT_{1A}	2.5	1.7	200	4.2	>10,000	780	98
5-HT_{1B}	1,800	810	49	2.5	>10,000	740	>10,000
5-HT_{1C}	6,600	>10,000	13	4.3	>10,000	3.3	2,000
5-HT_{1D}	930	160	690	3.5	>10,000	580	>10,000
5-HT_2	>10,000	4,500	780	1,300	1,400	9.1	0.71
5-HT_3	>10,000	>10,000	2,100	360	>10,000	360	>10,000
5-HT_{uptake}	560	>10,000	1,100	890	5.1	>10,000	1,600
trypt-amine	>10,000	>10,000	>10,000	1,600	>10,000	>10,000	>10,000

obtained either in the presence of 1 μM 5-HT or 1 μM eltoprazine, results in virtually blank images. The highest specific [^{3}H]-eltoprazine binding is found in substantia nigra, dorsal subiculum and basal ganglia (ventral pallidum and globus pallidus). Relatively high specific binding is also present in limbic structures, such as the dentate gyrus and CA_1-CA_2 region of the hippocampus, as well as in entorhinal cortex, lateral septum, central grey and dorsal raphe nucleus.

Areas containing moderate levels of [^{3}H]eltoprazine binding sites include the amygdala, the hypothalamus, the caudate putamen, the superior colliculus and the medial raphe nucleus. The cerebral cortex, thalamic nuclei and the cerebellum expressed only low densities of binding sites.

The overall distribution of [^{3}H]eltoprazine binding sites bears a strong resemblance to the location of 5-HT_1 binding sites labelled by [^{3}H]5-HT. Furthermore, [^{3}H]eltoprazine binding is reduced to levels of nonspecific binding by low concentrations (0.1 μM) of unlabelled 5-HT. Brain areas containing high amounts of 5-HT_{1B} sites (e.g. ventral pallidum and caudate putamen) exhibit relatively more [^{3}H]eltoprazine binding as compared to [^{3}H]5-HT binding, while relatively dense [^{3}H]5-HT binding is present in areas enriched in 5-HT_{1A} sites such as the lateral septum, and the internal layers of the

cerebral cortex (Fig. 1). Also, the choroid plexus of ventricles, which mainly contains 5-HT_{1C} sites, shows higher levels of [^{3}H]5-HT than of [^{3}H]eltoprazine binding.

Saturation analyses with [^{3}H]eltoprazine and [^{3}H]5-HT demonstrate high affinity binding of both ligands in many brain regions. Their apparent dissociation constants (K_D) and maximal binding capacities (B_{max}) in a few selected frontal brain areas are presented in Table 4. [^{3}H]5-HT has almost equal affinity for the various anatomical regions, whereas the K_D values of [^{3}H]eltoprazine are consistently higher in 5-HT_{1A} as compared to 5-HT_{1B} receptor dense areas.

TABLE 4

Saturation analysis of [^{3}H]5-HT and [^{3}H]Eltoprazine binding in coronal rat brain sections as determined by quantitative autoradiography

Brain areas	[^{3}H]5-HT		[^{3}H]Eltoprazine	
	K_D(nM)	B_{max}(fmol/mg)	K_D(nM)	B_{max} (fmol/mg)
5-HT_{1A} regions				
Parietal cortex lamina IV-VI	1.3 ± 0.24	57 ± 1.8	9.1 ± 0.99	55 ± 3.9
Septal nuclei	1.5 ± 0.26	319 ± 11.9	9.5 ± 0.73	278 ± 5.9
5-HT_{1B} regions				
Ventral pallidum	1.4 ± 0.31	298 ± 22.8	4.3 ± 0.42	337 ± 6.7
Caudate putamen	1.9 ± 0.28	72 ± 3.8	5.8 ± 0.65	79 ± 4.1

3.3 Effects on adenylate cyclase activity mediated by 5-HT_{1A} receptors

The forskolin-stimulated c-AMP production was studied in hippocampus slices of the rat. As illustrated in Fig. 2, the selective 5-HT_{1A} agonist 8-OH-DPAT induces a concentration-dependent inhibition of forskolin (10μM) stimulated adenylate cyclase activity. The maximal inhibition is 23 ± 3%, whereas 50% inhibition of the maximal effect (IC_{50}) occurs at 4 nM. The selective 5-HT_{1A} agonist flesinoxan induced an inhibition of adenylate cyclase activity comparable to 8-OH-DPAT. The maximal inhibition of flesinoxan was 24 ± 2% and the IC_{50} value 3 nM (see Fig. 2). In this functional model for 5-HT_{1A} receptor activity, eltoprazine inhibited adenylate

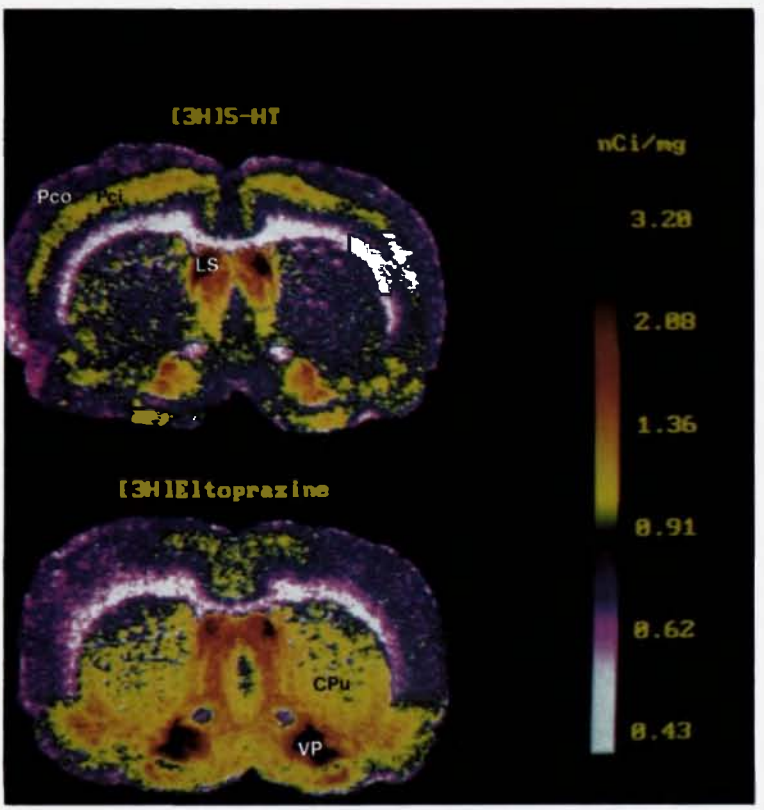

Fig. 1: Comparison between [^{3}H]5-HT and [^{3}H]eltoprazine binding sites in rat brain. The images presented are bright-field photographs of blown-up digitized and colour-coded autoradiograms generated from adjacent coronal brain sections (20 μM) incubated with either 1.8 nM [^{3}H]5-HT or 12 nM [^{3}H]eltoprazine. Sections were exposed to tritium sensitive films for 7 weeks. The optical density measurements were converted to nCi/mg tissue equivalent using ^{3}H-standard microscales (Amersham). CPu, caudate putamen; LS, lateral septum; Pci, parietal cortex (inner laminas IV-VI); Pco, parietal cortex (outer laminas I-III); VP, ventral pallidum.

cyclase activity, reaching significance only at the highest concentration tested (1 μM). The maximal response, however, is 16 ± 5%, which is 70% of the maximal effect obtained by 8-OH-DPAT.

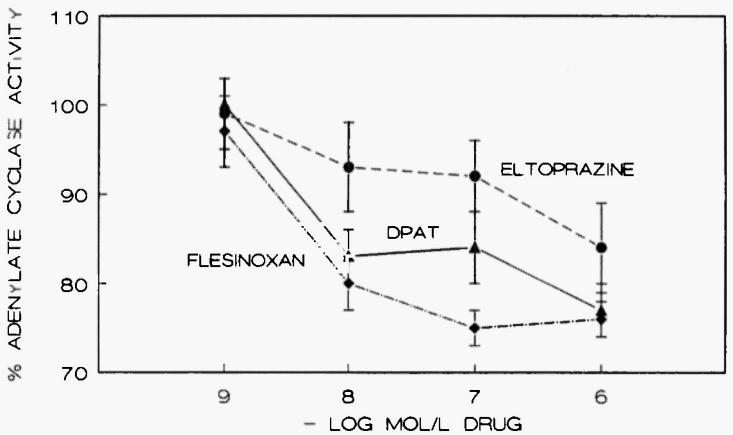

Fig. 2: Concentration curves of eltoprazine (•), 8-OH-DPAT (▲) and flesinoxan (▲) on inhibition of forskolin stimulated adenylate cyclase activity in rat hippocampus. Data are percentual means ± SEM (represented by vertical bars) of values obtained from 5-25 measurements.

3.4 Effects on 5-HT release mediated by 5-HT_{1B} autoreceptors

The K^+ stimulated release of [^{3}H]-5-HT from cortex slices is inhibited by exogenous 5-HT. As illustrated in Fig. 3, 5-HT induced a dose dependent inhibition, which resulted in a maximal inhibition of about 70% of the control values. Based on these dose response curves, the pD_2 value of 5-HT was estimated at 7.7 ± 0.1 in 10 independent experiments. Eltoprazine also induced an inhibition of the K^+ stimulated 5-HT release, but the maximal inhibition is much lower than that of 5-HT. The pD_2 value of eltoprazine was estimated at 7.8 ± 0.1 with an intrinsic activity (α) of 0.5 ± 0.1 based on 4 independent experiments. 8-OH-DPAT up to concentrations of 1 μM was inactive (see Fig. 3).

TFMPP inhibited the K^+ stimulated 5-HT release, with a similar affinity (pD_2 = 7.5 ± 0.1) as eltoprazine, but with a slightly higher intrinsic value (α = 0.7 ± 0.1).

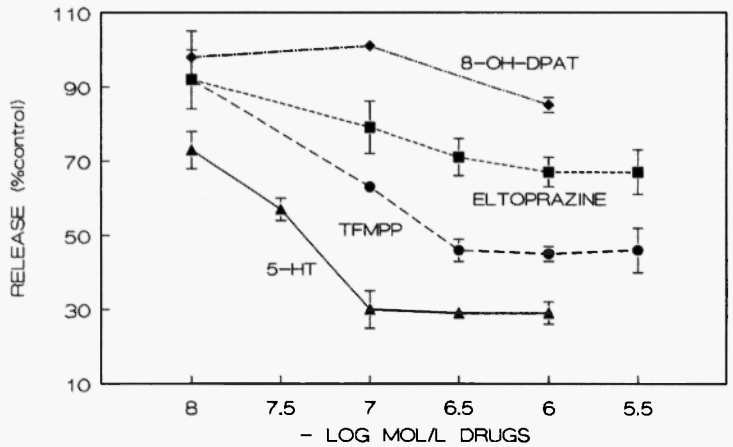

Fig. 3: Concentration curves of eltoprazine (■), 5-HT (▲), TFMPP (•) and 8-OH-DPAT (♦) on K^+ stimulated efflux of [^{3}H]5-HT from rat neocortex slices. Data are means ± SEM of values obtained from 4 measurements.

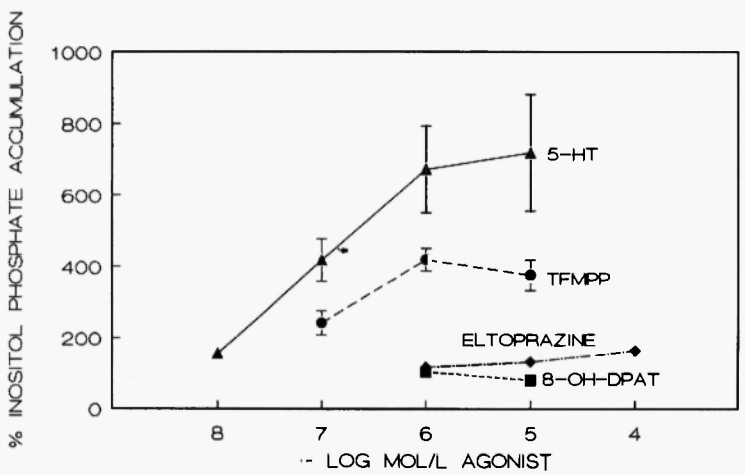

Fig. 4: Concentration curves of eltoprazine (♦), 5-HT (▲), TFMPP (•) and 8-OH-DPAT (■) on phosphatidyl inositol turnover in pig chorold plexus. The data are presented as % of stimulation above basal. Each point is the mean of 6 determinations. Vertical bars represent the SEM values.

3.5 Effects on phosphatidyl inositol turnover mediated by 5-HT_{1C} receptors

The choroid plexus of the pig contains a high density of 5-HT_{1C} receptors. These receptors are functionally linked to the phosphoinositol pathway. Stimulation of the 5-HT_{1C} receptors results in an increased PI turnover as measured by the inositol phosphate accumulation in the presence of lithium /25/. In our experiments, 5-HT induced a 7 fold increase in the inositol phosphate accumulation in the choroid plexus as illustrated in Fig. 4. In this functional 5-HT_{1C} model, eltoprazine and 8-OH-DPAT have no stimulatory effects on the PI turnover as shown in Fig. 4. This is in contrast to TFMPP which increased PI turnover about fourfold, suggesting (partial) agonistic properties in this model.

To evaluate the antagonistic properties on 5-HT_{1C} receptors, the effects of compounds were studied in the presence of 1 μM 5-HT. The 5-HT_{1C} antagonist mianserine reduced the 5-HT induced increase of PI turnover (see Fig. 5), with an IC_{50} of 0.05 μM. Spiperone, a compound with high affinity for the 5-HT_2 receptor, did not inhibit the 5-HT induced effects on PI turnover in concentrations up to 1 μM. Eltoprazine was able to inhibit the 5-

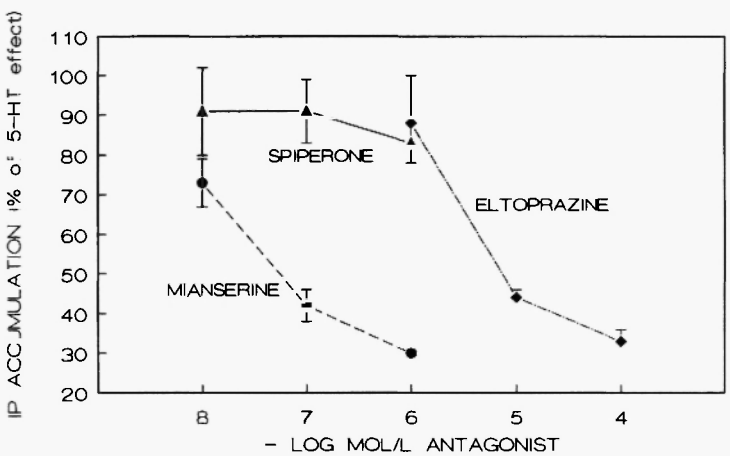

Fig. 5: Concentration curves of eltoprazine (♦), mianserine (•) and spiperone (▲) on 5-HT stimulated phosphatidyl inositol turnover in pig choroid plexus. The data are presented as the percentage of the maximal response occurring without antagonist added. Each point represents the mean of 6 determinations. The vertical bars represent the SEM values.

HT induced stimulation of PI turnover (see Fig. 6), but these antagonist properties occur at rather high concentrations (IC_{50} is 7 μM).

3.6 Effects on monoamine and metabolite levels in striatum of the rat

Considering the receptor-binding profile of eltoprazine and its functional activity on 5-HT_1 receptor subtypes, it was of interest to study its effects on the 5-HT system *in vivo*. As shown in Table 5, eltoprazine did not affect 5-HT levels in the striatum of the rat, but levels of its major metabolite 5-hydroxy indole acetic acid (5-HIAA) were decreased at doses of 10 mg/kg p.o. and higher. The decreased 5-HIAA levels without a change in 5-HT levels are generally found with 5-HT agonists and excludes MAO inhibiting or 5-HT releasing properties. At doses of 3 and 10 mg/kg, which are effective in inhibiting aggressive behaviour, eltoprazine has no significant effects on the DA system.

At high doses of 30 mg/kg p.o. eltoprazine shows no effect on DA levels, but a small increase in its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), is observed.

TABLE 5

Effects of eltoprazine on dopamine, serotonin and their metabolites in rat striatum

Dose	% of control (mean ± SEM)				
(mg/kg po)	DOPAC	HVA	DA	5-HT	5HIAA
Eltoprazine 0	100 ± 5	100 ± 5	100 ± 3	100 ± 4	100 ± 8
3	100 ±10	97 ± 9	101 ± 10	107 ± 7	91 ± 7
10	129 ±13	112 ± 9	88 ± 6	102 ± 4	74 ± 8*
30	166 ±18*	138 ± 16	88 ± 9	97 ± 4	82 ± 7*

* $p < 0.05$, Student t-test (2-tailed); the levels (ng/g tissue) in vehicle treated animals were for DOPAC: 464 ± 21; HVA: 756 ± 38; DA: 10446 ± 277; 5-HT: 378 ± 32; 5-HIAA: 260 ± 11.

3.7 Effects on 5-HT turnover in striatum of the rat

To further evaluate the serotonergic properties of eltoprazine *in vivo*, the effects on 5-HT turnover were studied. After inhibition of the aromatic L-amino-acid decarboxylase by NSD 1015, 5-hydroxytryptophan (5-HTP) accumulates in brain regions

containing 5-HT terminals. The rate of accumulation of 5-HTP has been used as an index of 5-HT turnover /70,71/.

Eltoprazine decreases 5-HTP accumulation as shown in Fig. 6 at doses as low as 0.3 mg/kg p.o. Maximal inhibition of 60% of the 5-HTP accumulation was obtained at oral doses of 3 mg/kg of eltoprazine. 8-OH-DPAT is more potent than eltoprazine in inhibiting the 5-HTP accumulation (Fig. 6), whereas TFMPP is slightly less potent than eltoprazine.

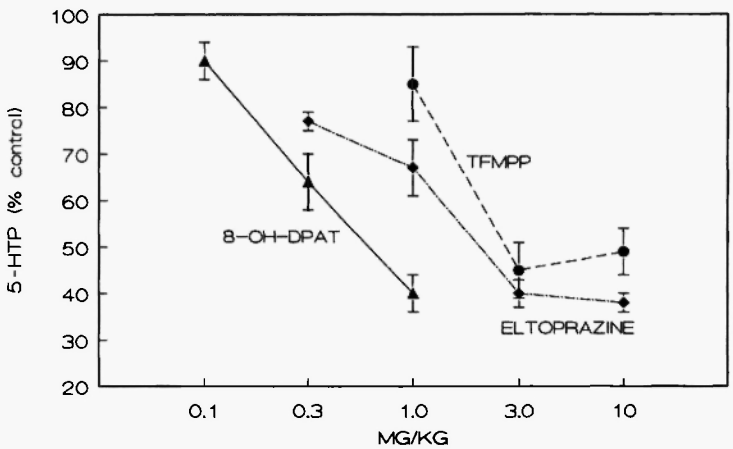

Fig. 6: Effects of eltoprazine (♦), TFMPP (•) and 8-OH-DPAT (▲) on 5-HTP accumulation in the striatum of rats. All animals received NSD 1015 (100 mg/kg) 30 min. before decapitation. Drugs were administered orally (except 8-OH-DPAT: i.p.) one hour before decapitation. The results are expressed as percentage of control levels. Data are means ± SEM of 5 observations in each group.

IV. DISCUSSION

Eltoprazine is a potent and selective inhibitor of the offensive components of agonistic behaviour in a number of animal paradigms /3/. In this report, we provide a neurochemical profile of this compound.

Virtually inactive on some twenty different receptor types, eltoprazine shows rather high affinity for a limited number of other receptors, notably for those belonging to the 5-HT_1-receptor subclass. With the exception of its relatively low affinity for 5-HT_{1D}

receptors, eltoprazine's receptor binding profile for the serotonergic receptors studied, rather closely resembles that of the natural ligand serotonin, albeit with approximately one tenth of the potency of the latter. Like serotonin, eltoprazine has 10 to 100 fold less affinity for the 5-HT_2 and 5-HT_3 receptor sites.

Eltoprazine must be classified as a selective 5-HT_1 ligand. Its selectivity either to other 5-HT-subpopulations or to receptors for which the compound has its next highest affinity, α_1- and $\beta_{1,2}$-adrenergic and dopamine-D_2 receptors, is an ample factor 10. It must be emphasized that eltoprazine's selectivity is considerably less pronounced than those of 8-OH-DPAT or flesinoxan, compounds which not only have a very high affinity for only one 5-HT_1 receptor, but which are selective by at least a factor of 100 with respect to all receptors measured.

In agreement with receptor binding studies in homogenates of rat frontal cortex, the autoradiographic data demonstrate high selectivity of eltoprazine for 5-HT_1 receptors, since low concentrations of 5-HT completely displace the specific [^{3}H]eltoprazine binding in all brain regions. The differential distribution of 5-HT_1 receptor subtypes may account for some of the discrepancies in binding patterns between 5-HT_1 receptors labelled with [^{3}H]5-HT and those labelled with [^{3}H]eltoprazine. The binding preference of [^{3}H]eltoprazine for 5-HT_{1B} receptor dense brain areas (e.g. the basal ganglia), as indicated by the relatively low K_D values in these areas, suggests a mild preference of eltoprazine for the 5-HT_{1B} receptor site. [^{3}H]5-HT, on the other hand, does not differentiate in its affinity for brain regions enriched in 5-HT_{1A} sites and regions primarily containing 5-HT_{1B} sites.

The lack of knowledge concerning the neuronal systems underlying offensive aggressive behaviour makes it difficult to link the [^{3}H]eltoprazine binding pattern with its anti-aggressive properties. The relatively high [^{3}H]eltoprazine binding in limbic brain structures (mainly to 5-HT_{1A} sites), as well as in subiculum, substantia nigra and basal ganglia (mainly to 5-HT_{1B} sites) opens the possibility of a neurochemical intervention in either motivational, integrative or even motor processes essential to offensive behaviour. One area of particular interest is the hypothalamus which contains a fair amount of 5-HT_{1B} sites and has been suggested to take part in the control of offensive aggression /71-73/. The finding that the attack behaviour of rats elicited by electrical hypothalamic stimulation can be inhibited by eltoprazine but not by the 5-HT_{1A} agonist 8-OH-DPAT suggests involvement of the 5-HT_{1B} receptor subtype /3/.

Needless to say, further investigations, such as local injections of

eltoprazine in specific rat brain regions, will be required to get a better insight into the neuronal substrates involved in the modulation of offensive agonistic behaviour.

The (ant)agonistic activity of eltoprazine on 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptor subtypes was evaluated in functional models. Many attempts have now been made to clarify the 5-HT_{1A} receptor-mediated response on a biochemical level. There is evidence that the 5-HT_{1A} binding site has a functional correlate in activating adenylate cyclase /19,74/. However, this increase in adenylate cyclase activity may be mediated by at least two subtypes of 5-HT_1 receptors /75/ or by a non-5-HT_1 receptor as suggested by Dumuis et al. /76/.

A more definite functional correlate of the 5-HT_1 receptor is the inhibition of forskolin-stimulated adenylate cyclase activity in hippocampus membranes /20/ and in cultured striatum and cortical neurons /21/. Various reports have shown that the maximal inhibitory effect is on average 20 to 30% /20,21/. In our experiments with hippocampus membranes, we obtained similar values for the maximal inhibition as can be obtained by full agonists such as 8-OH-DPAT and flesinoxan /77/. Eltoprazine also induced an inhibition of forskolin-stimulated adenylate cyclase activity, although the inhibition of eltoprazine was of lower amplitude than that of 8-OH-DPAT and flesinoxan. This suggests that eltoprazine may have a low potency at this receptor reaching maximal response above 1 μM. However, the affinity of eltoprazine for the 5-HT_{1A} site, as revealed by binding studies, makes this explanation unlikely. A more likely explanation is that eltoprazine is a partial agonist at this postsynaptic 5-HT_{1A} receptor site. The potency of eltoprazine is difficult to determine in terms of pD_2 values because of the rather small amplitude of inhibition, but assuming that the maximal response is obtained at 1 μM, the intrinsic activity is about 0.6.

The effects of eltoprazine on 5-HT_{1A} receptors have also been studied using electrophysiological techniques. Intracellular recordings in hippocampus slice preparation revealed a very small hyperpolarizing effect of micromolar concentrations of eltoprazine on CA1 pyramidal cells, presumably mediated by an increase in K^+ conductance /78/. These electrophysiological data suggest partial agonistic properties of eltoprazine, although the intrinsic activity seems to be much lower than on the adenylate cyclase response. Antagonistic properties of eltoprazine on this 5-HT_{1A} receptor mediated effect on K^+ channels were more evident.

Taken together, these *in vitro* data on 5-HT_{1A} receptor mediated effects suggest that eltoprazine has partial agonistic properties of which the intrinsic activity can vary between different models.

Whether differences in the concentration of 5-HT in the biophase in these models or whether different 5-HT_{1A} subtypes must be held responsible for the apparent differences in intrinsic activity remains to be established.

The 5-HT autoreceptor on the 5-HT terminals in the rat has been characterized as a 5-HT_{1B} receptor /30,31/. This autoreceptor modulates the 5-HT release from brain slices /28,29/. 5-HT agonists reduce the K^+ stimulated release of 5-HT from rat brain slices, which can be interpreted as a negative feedback signal. The 5-HT_{1B} properties of this autoreceptor are confirmed in our experiments by the inactivity of 8-OH-DPAT and the inhibitory effect of TFMPP, a 5-$HT_{1B/1C}$ agonist. Like 5-HT_{1B} agonists, eltoprazine also inhibited the K^+ stimulated 5-HT release. However, the maximal response was clearly less than that of the full agonist 5-HT. These data suggest partial agonistic activity of eltoprazine on the 5-HT_{1B} autoreceptor. This was further confirmed by experiments (data not shown) in which eltoprazine was able to antagonize part of the 5-HT effect.

The 5-HT_{1C} receptor site is coupled to the phosphatidyl inositol phosphate cycle /25,79/. The PI turnover in the choroid plexus which contains the highest density of 5-HT_{1C} sites in the brain /14,43/ has been suggested as a functional model for 5-HT_{1C} receptor mediated events /25/. Hoyer *et al.* /80/ have found that there is a highly significant correlation between the rank order of potency of agonists and antagonists and their affinities to 5-HT_{1C} sites. Although this receptor shows some resemblance to the 5-HT_2 receptor /80/, it clearly can be distinguished by the use of spiperone, which has a high affinity for the 5-HT_2 receptor, but virtually no effect on the 5-HT_{1C} mediated response, as illustrated in Fig. 5 /25,80/. Eltoprazine does not seem to have any intrinsic activity as an agonist on this receptor. This is in contrast to TFMPP, a structurally related compound, which shows (partial) agonistic properties in stimulating 5-HT_{1C} mediated phosphatidyl inositol hydrolysis. Eltoprazine can be characterized as an antagonist at the 5-HT_{1C} site, but its potency is considerably lower on the 5-HT_{1C} than on the 5-HT_{1A} or 5-HT_{1B} sites.

These results in the *in vitro* functional models indicate intrinsic activity at 5-HT_{1A} and 5-HT_{1B} receptors, but not at the 5-HT_{1C} subtype. Antagonistic properties of eltoprazine can be expected based on the partial agonistic effects. The intrinsic activity of eltoprazine on the various 5-HT subtypes is not the same; thus its outcome as an agonist or as an antagonist may depend on the relative occupation of these receptors by the endogenous ligand, i.e., 5-HT.

In vivo, acute administration of eltoprazine reduced 5-HIAA levels without change in the 5-HT levels in rat striatum. The decrease in the 5-HIAA/5-HT ratio indicates a reduced activity of the 5-HT neurons /81/ and suggests an agonistic action of eltoprazine. A reduced activity of 5-HT neurons has been observed for other 5-HT agonists as well /82-84/ and has been explained in terms of agonistic action at the 5-HT autoreceptor, either at the terminals or at a somadendritic level. The reduction in activity of 5-HT neurons is confirmed by the decreased accumulation of 5-HTP after decarboxylase inhibition. The 5-HTP accumulation reflects the tryptophan hydroxylase activity and it has been shown that this parameter can be used as a biochemical index for the activity of the 5-HT system /81/. The 5-HT synthesis rate is modulated by various 5-HT agonists. Both the 5-HT_{1A} agonists (e.g. 8-OH-DPAT) and the 5-HT_{1B} agonists (e.g. TFMPP) inhibit the 5-HT synthesis rate activity /83,85/. This fact points towards an involvement of these receptor subtypes in the modulation of the activity of the 5-HT neurons. The presence of somadendritic autoreceptors of the 5-HT_{1A} subtype /86,87/ and the presence of terminal autoreceptors of the 5-HT_{1B} subtype /30,31/ provides an explanation for this observation. Activation of both receptor subtypes may result in inhibition of the neuronal activity. Although these biochemical indices for 5-HT neuronal activity cannot be used to discriminate effects on different subtypes, they clearly point to a 5-HT agonistic action of eltoprazine.

In addition to the effects on the 5-HT system, the involvement of the DA system in the mechanism of action was studied. Based on the HPLC determinations of DA and its main metabolites, we can conclude that eltoprazine has some effect upon the DA neurons, but only at high doses. At doses at which the effects occur on aggression, eltoprazine has no effect on the DA neurons. This is in agreement with the receptor binding data and makes it unlikely that the DA system is involved in the anti-aggressive properties of eltoprazine.

In conclusion, from a variety of studies, described above, there is strong evidence that the pharmacological actions of eltoprazine can be attributed to 5-HT_1 receptors. The drug preferentially binds to 5-HT_{1A} and 5-HT_{1B} sites where it exerts agonistic actions. These results, together with the specific anti-aggressive properties of eltoprazine (Olivier, Mos and Rasmussen, this issue), correspond very well with the idea of an inverse relation between 5-HT and aggression /6,88/.

However, things are probably more complicated since eltoprazine behaves as a partial agonist, and both 5-HT_{1A} and 5-

HT_{1B} sites are located pre- as well as postsynaptically /13/. In this context it is interesting to mention that offensive behaviour in mice has been inhibited by both serotonergic agonists and antagonists /89-94/. Recent experiments in our laboratories revealed that depletion of brain 5-HT, by injections of 5,7-dihydroxytryptamine into the midbrain raphe nuclei, did not prevent the inhibitory effects of eltoprazine on offensive aggression (Sijbesma *et al.*, in preparation). This suggests that postsynaptic rather than presynaptic $5\text{-}HT_1$ sites mediate the behavioural actions of eltoprazine. Future experiments, with *in vivo* microdialysis and intracerebral administration of eltoprazine, may shed light on the way by which eltoprazine affects central serotonergic processes intrinsic to offensive agonistic behaviour.

V. REFERENCES

1. Olivier, B. and Mos, J. Serenics and aggression. *Stress Medicine* 1986; **2:** 197-209.
2. Olivier, B., Van Dalen, D. and Hartog, J. A new class of psychotropic drugs: Serenics. *Drugs Future* 1986; **11:**473-499.
3. Olivier, B., Van der Heyden, J., Schipper, J., Tulp, M.Th.M., Berkelmans, B. and Bevan, P. Serotonergic modulation of agonistic behaviour. In: Olivier, B., Mos, J. and Brain, P.F., eds., Ethopharmacology of agonistic behaviour in animals and humans. Dordrecht: Martinus Nijhoff 1987; 162-186.
4. Pucilowski, O. and Kostowski, W. Agressive behaviour and the central serotonergic systems. *Behav. Brain Res.* 1983; **9:** 33-48.
5. Miczek, K.A. and Donat, P. Brain 5-HT system and inhibition of aggressive behaviour. In: Bevan, P., Cools, A. and Archer, T., eds., Behavioural Pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Associates 1989; 117-144.
6. Valzelli, L. Psychopharmacology of aggression: An overview. *Int. Pharmacopsychiat.* 1981; **16:** 39-48.
7. Grant, E.C. and Macintosh, J.H. A comparison of the social postures of some common laboratory rodents. *Behaviour* 1963; **21:** 246-259.
8. Blanchard, R.J. and Blanchard, D.C. Aggressive behaviour in the rat. *Behav. Biol.* 1977; **21:** 197-224.
9. Timmermans, P.J.A. Social behaviour in the rat. PhD Thesis, University of Nijmegen, The Netherlands, 1978.
10. Adams, D.B. Brain mechanisms for offense, defense and submission. *Behav. Brain Sci.* 1979; **2:** 201-241.
11. Albert, D.J. and Walsh, M.L. Neural systems and the inhibitory modulation of agonistic behaviour: a comparison of mammalian species. *Neurosci. Biobehav. Rev.* 1984; **8:** 5-24.
12. Steinbusch, H.W.M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat – Cell bodies and terminals. *Neurosci.* 1981; **6:** 557-618.

13. Peroutka, S.J. 5-Hydroxytryptamine receptor subtypes: molecular, biochemical and physiological characterization. *Tr. Neurosci.* 1988; **11:** 496-500.
14. Pazos, A. and Palacios, J.M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.* 1985; **346:** 205-230.
15. Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* 1986; **25:** 563-576.
16. Hoyer, D., Engel, G. and Kalkman, H.O. Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, [^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserine. *Eur. J. Pharmacol.* 1985; **118:** 13-23.
17. Heuring, R.E. and Peroutka, S.J. Characterisation of a novel [^{3}H]-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* 1987; **7:** 894-903.
18. Shenker, A., Maayani, S., Weinstein, H. and Green, J.P. Two 5-HT receptors linked to adenylate cyclase in guinea pig hippocampus are discriminated by 5-carboxamidotryptamine and spiperone. *Eur. J. Pharmacol.* 1985; **109:** 427-429.
19. Markstein, R., Hoyer, D. and Engel, G. 5-HT_{1A}-receptor mediated stimulation of adenylate cyclase in rat hippocampus. *Naunyn-Schmiedeb. Arch. Pharmacol.* 1986; **333:** 335-341.
20. Devivo, M. and Maayani, S. Characterization of the 5-hydroxytryptamine-1A receptor mediated inhibition of forskolin stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *J. Pharm. Exp. Ther.* 1986; **238:** 248-253.
21. Weiss, S., Sebben, M., Kemp, D.E. and Bockaert, J. Serotonin 5-HT_1 receptors mediate inhibition of cyclic AMP production in neurons. *Eur. J. Pharmacol.* 1986; **120:** 227-230.
22. Bouhelal, R.L., Smounya, K. and Bockaert, J. 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra. *Eur. J. Pharmacol.* 1988; **151:** 189-196.
23. Schoeffter, P., Waeber, C., Palacios, J.M. and Hoyer, D. The 5-hydroxytryptamine 5-HT_{1D} receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. *Naunyn-Schmiedeb. Arch. Pharmacol.* 1988; **337:** 602-608.
24. Palacios, J.M., Markstein, R. and Pazos, A. Serotonin 5-HT_{1C} sites in the choroid plexus are not linked in a stimulatory or inhibitory way to adenylate cyclase. *Brain Res.* 1986; **380:** 151-154.
25. Conn, J.P., Sanders-Bush, E., Hoffman, B.J. and Hartig, P.R. A unique receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci.,* 1986; **83:** 4086-4088.
26. Conn, P.J. and Sanders-Bush, E. Serotonin stimulated phosphoinositide turnover; mediation by the S2 binding site in the cerebral cortex but not in subcortical regions. *J. Pharm. Exp. Ther.* 1985; **234:** 195-203.
27. Derkach, V., Surprenant, A. and North, R.A. 5-HT_3 receptors are membrane ion channels. *Nature* 1989; **339:** 706-709.

28. Göthert, M. and Weinheimer, G. Extracellular 5-hydroxytryptamine inhibits 5-hydroxytryptamine release from rat brain cortex slices. *Naunyn Schmiedeb. Arch. Pharmacol.* 1979; **310:** 93-96.
29. Baumann, P.A. and Waldmeier, P.C. Further evidence for negative feedback control of serotonin release in the central nervous system. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1981; **317:** 36-43.
30. Engel, G., Gothert, M., Hoyer, D., Schlicker, E. and Hillenbrand, K. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1986; **332:** 1-7.
31. Maura, G., Roccatagliata, E. and Raiteri, M. Serotonin autoreceptor in rat hippocampus: pharmacological characterization as a subtype of the 5-HT_1 receptor. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1986; **334:** 323-326.
32. Middlemiss, D.N. 8-Hydroxy-2-(dipropylamino)tetralin is devoid of activity at the 5-hydroxytryptamine receptor in the rat brain. Implications for the proposed link between the autoreceptor and the [^{3}H]5-HT recognition site. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1984; **327:** 18-28.
33. Schlicker, E., Fink, K., Gothert, M., Hoyer, D., Molderings, G., Roschke, I. and Schoeffter, P. The pharmacological properties of the presynaptic serotonin autoreceptor in the pig brain cortex conform to the 5-HT_{1D} receptor subtype. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1989; **340:** 45-51.
34. Middlemiss, D.N., Bremer, M.E. and Smith, S.M. A pharmacological analysis of the 5-HT receptor mediating inhibition of 5-HT release in the guinea-pig frontal cortex. *Eur. J. Pharmacol.* 1988; **157:** 101-107.
35. Schipper, J. and Tulp, M.Th.M. Serotonin autoreceptors in guinea pig cortex slices resemble the 5-HT_{1D} binding site. *Abstr. Soc. Neuroscience* 1988; **14:** 552.
36. Timmermans, P.B.M.W.N., Kwa Ali, F. and Schoop, A.M.C. Identical antagonist selectivity of central and peripheral alpha_1-adrenoceptors. *Mol. Pharmacol.* 1981; **20:** 295-301.
37. U'Pritchard, D.C., Greenberg, D.A. and Snyder, S.H. Binding characteristics of a radiolabelled agonist and antagonist at central nervous system alpha noradrenergic receptors. *Mol. Pharmacol.* 1977; **13:** 455-473.
38. Nahorski, S. Heterogeneity of cerebral adrenoceptor binding sites in various vertebrate species. *Eur. J. Pharmacol.* 1978; **51:** 199-209.
39. Burt, D.R., Creese, I. and Snyder, S.H. Properties of [^{3}H]-haloperidol and [^{3}H]-dopamine binding associated with dopamine receptors in calf brain membranes. *Mol. Pharmacol.* 1976; **12:** 800-812.
40. Creese, I., Schneider, R. and Snyder, S.H. [^{3}H]-Spiroperidol labels dopamine receptors in rat pituitary and brain. *Eur. J. Pharmacol.* 1977; **46:** 377-381.
41. Gozlan, H., Mestikawy, S.El., Glowinsky, J. and Hamon, M. Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. *Nature* 1983; **305:** 140-142.
42. Sills, M.A., Wolfe, B.B. and Frazer, A. Determination of selective and non-selective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. *J. Pharmacol. Exp. Ther.* 1984; **231:** 480-487.
43. Pazos, A., Hoyer, D. and Palacios, J.M. The binding of serotonergic ligands to porcine choroid plexus: characterisation of a new type of serotonin recognition site. *Eur. J. Pharmacol.* 1985; **106:** 539-546.

44. Creese, I. and Snyder, S.H. [^{3}H]-Spiroperidol labels serotonin receptors in rat cerebral cortex and hippocampus. *Eur. J. Pharmacol.* 1978; **49:** 201-202.
45. Hoyer, D. and Neijt, H.C. Identification of serotonin 5-HT_3 recognition sites by radioligand binding in NG108-15 neuroblastoma-glioma cells. *Eur. J. Pharmacol.* 1987; **143:** 291-293.
46. Habert, E., Graham, D., Hahraoui, L., Claustre, Y. and Langer, S.Z. Characterization of [^{3}H]-paroxetine binding to rat cortical membranes. *Eur. J. Pharmacol.* 1985; **118:** 107-114.
47. Graham, D. and Langer, S.Z. [^{3}H]-tryptamine binding sites of rat cerebral cortex: pharmacological profile and plasticity. *Neuropharmacology* 1987; **26:** 1093-1097.
48. Tran, Y.T., Chang, R.S.L. and Snyder, S.H. Histamine-H_1 receptors identified in mammalian brain membranes with [^{3}H]-mepyramine. *Proc. Natl. Acad. Sci. U.S.A.* 1978; **75:** 6290-6294.
49. Doods, H. Affinity and selectivity of muscarinic antagonists for the M_1, M_2 and M_3 binding sites. In: Doods, H.N. Characterisation and classification of muscarinic receptors and their subtypes, PhD Thesis, University of Amsterdam, The Netherlands, 1987; 40-53.
50. Pert, C.B. and Snyder, S.H. Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.* 1974; 868-879.
51. Hiller, J.M. and Simon, E.J. Specific high affinity [^{3}H]ethylketocyclazocine binding in rat central nervous system: lack of evidence for k-receptors. *J. Pharmacol. Exp. Ther.* 1980; **214:** 516-519.
52. Leslie, F.M., Chavkin, C. and Cox, B.M. Opioid binding properties of brain and peripheral tissues: evidence for heterogeneity in opioid ligand binding sites. *J. Pharmacol. Exp. Ther.* 1980; **214:** 395-402.
53. Ehlert, F.J., Roeske, W.R., Itoga, E. and Yamamura, H.I. The binding of [^{3}H]-nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex and ileum of rats. *Life Sci.* 1982; **30:** 2191-2202.
54. Reynolds, I.J., Snowman, A.M. and Snyder, S.H. (-)-[^{3}H]-Desmethoxyverapamil labels multiple calcium channel modulator receptors in brain and skeletal muscle membranes: differentiation by temperature and dihydropyridines. *J. Pharmacol. Exp. Ther.* 1986; **237:** 731-738.
55. Braestrup, C. and Squires, R.F. Pharmacological characterisation of benzodiazepine receptors in the brain. *Eur. J. Pharmacol.* 1978; **48:** 263-270.
56. Herschel, M. and Baldessarini, R.J. Evidence for two types of binding of [^{3}H]-GABA and [^{3}H]-muscimol in rat cerebral cortex and cerebellum. *Life Sci.* 1979; 1849-1854.
57. Müller, W.E. and Snyder, S.H. Strychnine binding associated with synaptic glycine receptors in rat spinal cord membranes: ionic influences. *Brain Res.* 1978; **147:** 107-116.
58. Murphy, D.E., Hutchinson, A.J., Hurt, S.D., Williams, M. and Sills, M.A. Characterisation of the binding of [^{3}H]CGS 19755: a novel N-methyl-D-aspartate antagonist with nanomolar affinity in rat brain. *Br. J. Pharmacol.* 1988; **95:** 932-938.
59. Mong, S., Wu, H.L., Hogaboom, G.K., Clark, M.A. and Crooke, S.T. Characterisation of the leukotriene-D4 receptor in guinea-pig lung. *Eur. J. Pharmacol.* 1984; **102:** 1-11.

60. Largent, B.L., Wikström, H., Gundlach, A.L. and Snyder, S.H. Structural determinants of σ receptor affinity. *Mol. Pharmacol.* 1987; **32:** 772-784.
61. Taylor, R.L. and Burt, D.R. Preparation of 3H-[3-Me-His2] as an improved ligand for TRH receptors. *Neuroendocrinol.* 1981; **32:** 310-316.
62. Van Dijk, A., Richards, J.G., Trzeciak, A., Gillessen, D. and Möhler, H. Cholecystokinin receptors: biochemical demonstration and autoradiographical localisation in rat brain and pancreas using [^{3}H]-cholecystokinin-8 as radioligand. *J. Neurosci.* 1984; **4:** 1921-1933.
63. Perrone, M.H., Diehl, R.E. and Haubrich, D.R. Binding of [^{3}H]-substance P to putative substance P receptors in rat brain membranes. *Eur. J. Pharmacol.* 1983; **95:** 131-133.
64. Sijbesma, H., Schipper, J. and De Kloet, E.R. Eltoprazine, a drug which reduces aggressive behaviour, binds selectively to 5-HT$_1$ receptor sites in the rat brain: An autoradiographic study. *Eur. J. Pharmacol.* 1990; **177:** 55-66.
65. Paxinos, G. and Watson, C. The Rat Brain in Stereotaxic Coordinates. New York: Academic Press, 1986.
66. Munson, P.J. and Rodbard, D. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 1980; **107:** 220-239.
67. Salomon, Y. Adenylate cyclase assay. In: Brooker, G., Greengard, P. and Robison, G.A., eds., Advances in Cyclic Nucleotide Research, Vol. 10. New York: Raven Press, 1979; 35-55.
68. Berridge, M.J., Dawson, C., Downesm, C.P., Heslop, J.P. and Irvine, R.F. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem. J.* 1983; **212:** 473-482.
69. Plantje, J.F., Steinbusch, H.W.M., Schipper, J., Dijks, F.A., Verheyden, P.F.H.M. and Stoof, J.C. D$_2$ dopamine receptors regulate the release of ^{3}H-dopamine in rat cortical regions showing dopamine immunoreactive fibers. *Neuroscience* 1987; **20:** 157-168.
70. Carlsson, A., Bédard, P., Lindqvist, M. and Magnusson, T. The influence of nerve-impulse flow on the synthesis and metabolism of 5-hydroxytryptamine in the central nervous system. *Biochem. Soc. Symp.* 1972; **36:** 17-32.
71. Bermond, B. Effects of medial preoptic hypothalamus anterior lesions on three kinds of behaviour in the rat: Intermale aggressive, male-sexual, and mouse-killing behaviour. *Aggr. Behav.* 1982; **8:** 335-354.
72. Kruk, M.R., Van der Laan, C.E., Meelis, W., Phillips, R.E., Mos, J. and Van der Poel, A.M. Brain-stimulation induced agonistic behaviour: A novel paradigm in ethopharmacological aggression research. In: Miczek, K.A., Kruk, M.R. and Olivier, B., eds., Ethopharmacological aggression research. New York: Alan R. Liss, 1984; 157-177.
73. Vergnes, M., Depaulis, A., Boehrer, A. and Kempf, E. Selective increase of offensive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. *Behav. Brain Res.* 1988; **29:** 85-91.
74. Fayolle, C., Fillion, M.P., Barone, P.B., Oudar, P., Rousselle, J.C. and Fillion, G. 5-Hydroxytryptamine stimulates two distinct adenylate cyclase activities in rat brain: high-affinity activation is related to a 5-HT1 subtype different from 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$. *Fund. Clin. Pharmacol.* 1988; **2:** 195-214.
75. Shenker, A., Maayani, S., Weinstein, H. and Green, J.P. Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate

cyclase in guinea pig hippocampal membranes. *Mol. Pharmacol.* 1987; 357-367.

76. Dumuis, A., Bouhelal, R., Sebben, M., Cory, R. and Bockaert, J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.* 1988; **34:** 880-887.
77. Schoeffter, P. and Hoyer, D. Centrally acting hypotensive agents with affinity for 5-HT_{1A} binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. *Br. J. Pharmacol.* 1988; **95:** 975-985.
78. Joëls, M., Pennartz, C.M.A., Sijbesma, H. and Schipper, J. Eltoprazine suppresses hyperpolarizing responses to serotonin in rat hippocampus. *J. Pharmacol. Exp. Ther.* [in press].
79. Hoyer, D. Molecular pharmacology and biology of 5-HT_{1C} receptors. *TIPS* 1988; **9:** 89-93.
80. Hoyer, D., Waeber, C., Schoeffter, P., Palacios, J.M. and Dravid, A. 5-HT_{1C} receptor mediated stimulation of inositol phosphate production in pig choroid plexus. A pharmacological characterization. *Naunyn-Schmiedeb. Arch. Pharmacol.* 1989; **339:** 252-258.
81. Shannon, N.J., Gunnet, J.W. and Moore, K.E. A comparison of biochemical indices of 5-hydroxytryptaminergic neuronal activity following electrical stimulation of the dorsal raphe nucleus. *J. Neurochem.* 1986; **47:** 958-965.
82. Hutson, P.H., Dourish, C.T. and Curzon, G. Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors. *Eur. J. Pharmacol.* 1986; **129:** 347-352.
83. Hjorth, S., Carlson, A., Lindberg, P., Sanchez, D., Wikstrom, H., Arvidsson, L.E., Hacksell, U. and Nilsson, J.L.G. 8-Hydroxy-2-(dipropylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J. Neur. Transm.* 1982; **55:** 169-181.
84. Hamon, M., Fattaccini, C.M., Adrien, J., Gallissot, M.C., Martin, P. and Gozlan, H. Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine$_{1A}$ agonists with potential anxiolytic properties. *J. Pharmacol. Exp. Ther.* 1988; **246:** 745-752.
85. Fuller, R.W., Snoddy, H.D., Mason, N.R. and Malloy, B.B. Effect of 1-(m-trifluoromethylphenyl)-piperazine on ^{3}H-serotonin binding to members from rat brain in vitro and on serotonin turnover in rat brain in vivo. *Eur. J. Pharmacol.* 1978; **52:** 11-18.
86. Sprouse, J.S. and Aghajanian, G.K. (-)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_{1A} selective agents. *Eur. J. Pharmacol.* 1986; **128:** 295-298.
87. Dourish, C.T., Hutson, P.H. and Curzon, G. Putative anxiolytics 8-OH-DPAT, buspirone and TVXQ 7821 are agonists at 5-HT_{1A} autoreceptors in the raphe nuclei. *Tr. Pharmacol. Sci.* 1986; **7:** 212-214.
88. Vergnes, M., Depaulis, A. and Boehrer, A. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. *Physiol. Behav.* 1986; **36:** 653.
89. Malick, J.B. and Barnett, A. The role of serotonergic pathways in isolation-induced aggression in mice. *Pharmacol. Biochem. Behav.* 1976; **5:** 55-61.
90. Weinstock, M. and Weiss, C. Antagonism by propranolol of isolation induced aggression in mice: Correlation with 5-hydroxytryptamine receptor blockade. *Neuropharmacology* 1980; **19:** 653-656.

91. Winslow, J.T. and Miczek, K.A. Habituation of aggression in mice: Pharmacological evidence of catecholaminergic and serotonergic mediation. *Psychopharmacology* 1983; 81: 286-291.
92. Lindgren, T. and Kantak, M. Effects of serotonin receptor agonists and antagonists on offensive aggression in mice. *Aggr. Behav.* 1987; **13:** 87-96.
93. McMillen, B.A., DaVanzo, E.A., Scott, S.M. and Song, A.H. N-alkyl-substituted aryl-piperazine drugs: Relationship between affinity for serotonin receptors and inhibition of aggression. *Drug Dev. Res.* 1988; **12:** 53-62.
94. Olivier, B., Mos, J., Van der Heyden, J. and Hartog, J. Serotonergic modulation of social interactions in isolated male mice. *Psychopharmacology* 1989; **97:** 154-156.