THE PSYCHOPHARMACOLOGY OF SEROTONIN

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SUMMARY

The present paper gives, after a short overview of the state of the art of the serotonergic system in the CNS, several examples of functional approaches to study the effects of 5-HT drugs.

Temperature regulation is an important physiological system which is heavily influenced by serotoninergic neurotransmission. One particular animal model of stress-induced hyperthermia is explained to illustrate the anti-stress (anxiolytic) effects of 5HT1a-agonist, but not SSRI's or 5-HT3 antagonists. Then the effects of serotonin agonists on behaviour are described, exemplified by the serotonergic syndrome.

Lower Lip retraction is very characteristic for activation of the 5-HT1a receptor, wet dog shaking for 5-HT2 (A) receptor activation whereas purposeless chewing is associated with activation of 5-HT2(C) receptors. In an animal model reflecting anxiety, ultrasonic distress calls in rat pups, 5-HT1A agonists, like benzodiazepines are potent anxiolytic. Also the SSRI's have aniolytic properties, but not the 5-HT3 receptor antagonists. 5-HT2A/2C antagonists have anxiogenic properties in this paradigm. In an animal model of depression, forced swimming in rats, 5-HT1A agonists appear to exert strong antidepressant activity, like the classical tricyclic antidepressants. The SSRI's show up as false negatives in this paradigm, i.e. they have no activity whereas they are clinically proven antidepressants. In an animal model of aggression, resident-intruder aggression in rats, in particular drugs with agonistic activity at the 5-HT1B receptor exert very specific anti-aggressive effects. Drug-discrimination techniques have been employed to illustrate an "in vivo" mechanism of action paradigm. It appears possible both in rats and pigeons, to describe very specific cues (drug stimuli) for the 5-HT 1A and the 5-HT1B receptor using flesinoxan (5-HT1A) and eltoprazine (mixed 5-HT1A/1B) as training drugs. Finally, it is attempted to summarize the clinical stage of the serotonergic field.

Keyword:serotonin-temperature-anxiety-depression-aggression-5-HT1A-agonists-SSRI-benzodiazepines.

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INTRODUCTION

Serotonin (5-HT) is a neurotransmitter which was already discovered many decades ago. Already in the early fifties and sixties suggestions were made about its involvement in various psychiatric diseases, including psychosis, anxiety and depression.

This was further substantiated in the eighties and early nineties, where it became clear that 5-HT is also involved in an elaborate complex of psychiatric disorders, e.g., obsessive compulsive disorder, alcohol and drug abuse, sexual disorders, aggression and feeding disorders. Serotonin is localized for less than 5% in the brain and the rest in the remaining parts of the body, e.g., the blood platelets and the gut. The serotonin in the CNS

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platelets and the gut. The serotonin in the CNS does however play a very important role in behaviour.

Serotonergic cells are located in the raphe nuclei in the midbrain. Both ascending and descending projections are present. The ascending part projects to a wide array of brain structures, but in particular to limbic areas. The last decade showed an increasing number of serotonergic receptors, present on pre-and postsynaptic localisations in the CNS.

SEROTONIN RECEPTOR SUBTYPES

At present in the serotonin receptor family 8 subfamilies of serotonin receptors are distinguished (92), viz. 5-HT1A, 5-HT1E, 5-HT1F, 5-HT2, 5-HT3, 5-HT4 and 5-HT5. The various subfamilies may consist of different subtypes, e.g. the subfamily 5-HT1D comprises the 5-HT1B, 5-HT1D and 5-HT1D? receptors, the 5-HT2 subfamily the 5-HT2A (former 5-HT2), 5-HT2B and 5-HT2C (former 5-HT1C) receptors (42). In this article the 5-HT1C and 5-HT2C, like the 5-HT2 and 5-HT2A are used interchangeable, because a lot of pharmacology is still based on old terminology. The different receptors are neuroanatomically localized at different sites in the CNS (76), e.g. 5-HT1A receptors are abundantly present in the hippocampus, septum, neocortex and raphe nuclei, 5-HT1B/1D receptors in the pallidum and substantia nigra, 5-HT1C (2C) receptors in the hypothalamus, pallidum, substantia nigra and particularly in the choroid plexus, 5-HT2 (2A) receptors in the neocortex and 5-HT3 receptors abundantly in the substantia gelatinosa. The distribution of 5-HT4 receptors in the CNS is incompletely known although preliminary evidence showed high binding in the striatum, olfactory tubercle, nucleus accumbens, globus pallidus and substantia nigra (35). 5-HT5 receptors occur prdominantly in the cerebral cortex, hippocampus, habunela, olfactory bulb and cerebellum (78). Moreover, 5-HT1A receptors are localized pre-and postsynaptically, and 5-HT2C (1C), 5-HT2A (2) 5-HT2A (2) and 5-HT3 receptors presumably postsynaptically (fig. 1).

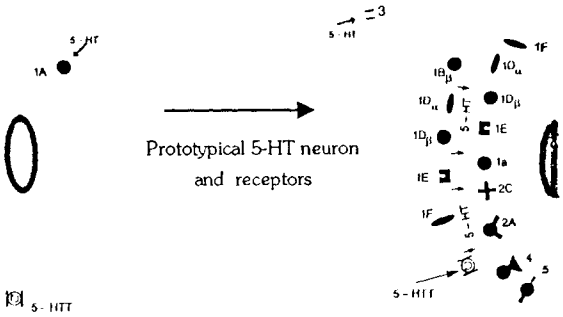

Fig. 1 Schematic portrayal of the various serotonergic receptors on a serotonergic neuron and a postsynaptic cell.

The 5-HT receptor family belongs to two extended gene superfamilies: The G-protein coupled receptor superfamily and the ligand-gated ion-channel superfamily. The 5-HT1, 5-HT2, 5-HT4 and 5-HT5 receptors are linked to the modulation of either adenylate cyclase or modulation of phosphoinositol turnover via G-proteins, whereas 5-HT3 receptors modulate an ion-channel. Recently the 5-HT transporter has been cloned in rats (10, 39), and humans (51); its structure revealed a 12 transmembrane domain build-up. 5-HT transporters are localized both on the presynaptic part of the terminal but also on the cell bodies of 5-HT neurons in the raphe nuclei (22, 25, 41).

It is evident that the different 5-HT receptors are extremely heterogeneus with regard to anatomical localization, function and coupling to second messenger systems and it may be expected that the functional effects of activation and inhibition of the various receptors is rather heterogeneous too.

An important technical aspect of studying the influence of a particular 5-HT receptor in behaviour, is the availability of specific ligands. Table 1 summarizes the current ligands presently used in 5-HT research.

Although the data in this table are in vitro receptor affinities, they clearly give an important first indication of the profile of a drug. Thus, specific 5-HT1A receptor agonists are available, but specific 5-HT1A receptor antagonists are lacking (NAN-190, pindolol, propranolol, cyanopindolol) orjust recently becoming available (WAY 100, 169;

SDZ216-525 (83) (methyl 4-(4-(4- (1, 1, 3-trioxo-2H-1, 2 - benzoisothiazol -2 yl) butyl-1 piperazine) 1H-indole-2-carboxylate); (S)-UH301 ((S)--fluoro-8-hydroxy-2- (dipropylamino) tetralin)). No specific 5-HT1B, 5-HT1D or 5-HT2 ligands are available, whereas very specific 5-HT3 receptors antagonists are present. For the more recently found receptors (5-HT1D , 5-HT1D, 5-HT1E, 5-HT1F and 5-HT5) no spesific ligands are available yet.

This means that behavioural effects induced by certain ligands must be considered carefully, because they may be caused by multi-receptor activation, blockade or interaction.

FUNCTIONAL APPROACHES TO THE STUDY OF SEROTONERGIC AGENTS

Various approaches can be taken to study the functional effects of serotonergic agents. Physiological and endocrinological parameters or behavioural models (both contitioned and unconditioned) may be used in studying the functional roles of the various 5-HT receptors. This paper will not review all functional models, but will focus on relevant examples.

A physiological model, frequently used to characterize serotonergic (but also other pharmacological) mechanisms of action is temperature modulation in either mice or rats, whereas some behaviour models can be used as behavioural "bioassays".

Moreover, various behavioural approaches are used as therapeutic tests, e.g. animal models of anxiety, depression, aggression, etc. Examples of the various approaches will be outlined in the following part of this chapter.

TEMPERATURE REGULATION

An extensive literature exists about the influence of serotonergic ligands on temperature in rodents. Serotonergic drugs produce varying effects on thermoregulation critically depending upon the dose, species and ambient temperature. Besides peripheral, also central 5-HT mechanisms are involved in thermoregulation; an important area in the CNS seems to be the anterior hypothalamus(23).

5-HT1A receptors are involved in temperature regulation, because 5-HT1A receptor agonists like 8-OH-DPAT lower body temperature in rats and mice, which effect can be antagonized by 5-HT1A receptor antagonists ((-) pindolol, (-) propranolol). There is however quite some controversy whether the hypothermic effect is mediated via the postsynaptic or the somatodendritic autoreceptor; a further complication seems the rat/mice difference in this aspect (9, 54, 55, 63). The situation for other 5-HT receptors is not less complicated. TFMPP and mCPP, agonists at 5-HT1C(2C) and (less at) 5-HT1B receptors, also induce hypothermia (52). However, when measured under high temperature conditions, these drugs induce hyperthermia (47), which is suggested to be mediated via 5-HT2 (2A)/5-HT1C(2C) receptors.

Several factors appear to play a role in the modulation of body temperature, including ambient temperature (34), way of housing, species, sex (20) and strain differences (44) and light-dark cycle (46).

Fig. 2 shows the effects of a subcutaneous injection with saline or 1 mg/kg 8-OH-DPAT on the core temperature (abdominal thermistor) of isolated male rats using a telemetric measurement system. A saline injection does not change the core temperature, whereas 8-OH-DPAT reduces it by approx. 3°C and its effect is relatively long-lasting. A similar profile was found after flesinoxan (3 mg/kg sc). This illustrates the potent hypothermic effect of 5-HT1A receptor agonists in isolated rats.

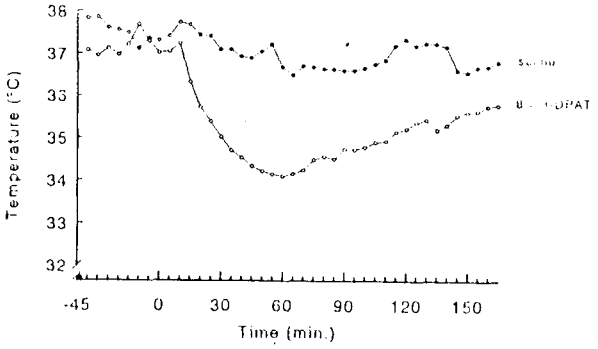

Fig. 2 Effects of 8-OH-DPAT (1mg/kg, i.p.) and saline (2 ml/kg, i.p.) on body temperature of rats. The thermistor is placed in the abdominal cavity. One rat received saline and the other 8-OH-DPAT at t=0 min.

Although in this experiment no real effect after saline injection was found, Dilsaver and Majchrzak (26) report that an i.p. saline injection

leads to a small increase in core temperature (around 0.5-0.6°C) compared to 0.13°C when animals were only disturbed. These and other data (12, 19, 77, 84) illustrate that mild stress can raise the body temperature of rats.

An interesting paradigm which includes these factors is stress-induced hypethermia in mice (SIH). It is wellknown that anticipation of an aversive event leads to an enhancement of body temperature. This stress-related anticipatory anxiety seems to be universal, including mice (15), rats (19), rabbits (86) and humans (53). When group-housed mice are removed one-by-one from their home cage, mice removed last always have higher temperatures than those removed first. We used cages containing 10 male mice, which were measured successively with a 1 min interval.

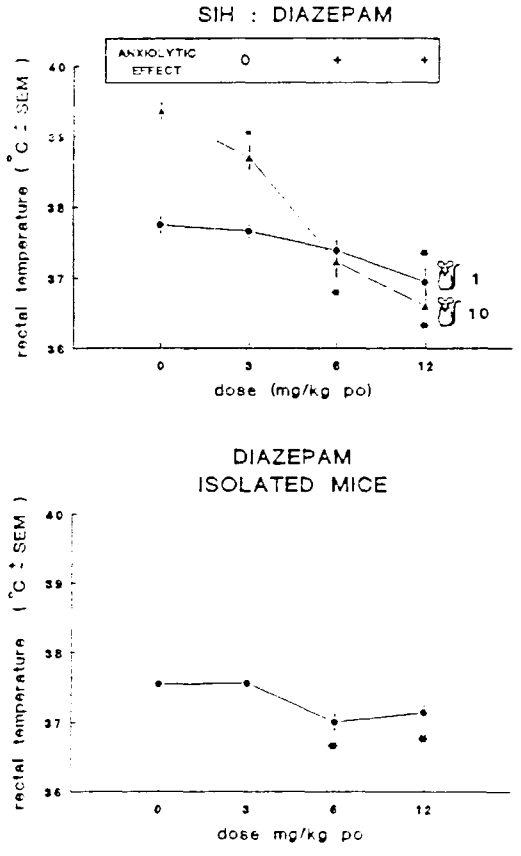

Fig. 3 Rectal temperature of male mice housed in a cage with 10 mice each (8 cages). Each minute the rectal temperature of a mouse was measured so that all ten mice were measured sequentially. The mean data on these mice nrs. 1 to 10 (N=8 for each point) are shown (±SEM). Sequential temperature measurement leads to hyperthermia which is maximal around min 8(mouse nr. 7).

Fig 3. shows the effeccts of measuring the temperature in 8 cages, each containing 10 mice. As one can see the stress-induced hyperthermia is rapidly occurring, in that already mouse 2 shows an increase, which is maximally around mice 8 to 10. Extensive work (104) has shown that increasing that increasing the interval between rectal temperature measurementt leads to similar results, i.e. the maximum rise occurs in about 8-10 minutes, whereas the duration of this hyperthermia is at least 30 minutes, returning to basal temperture after 60 minutes.

Fig 4. illustrates the basic pharmacological findings of this paradigm, using diazepam as a typical example.

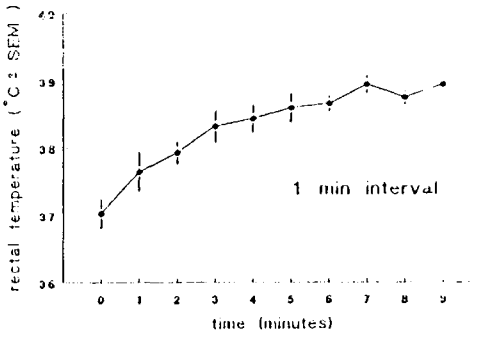

Fig 4 The effects of diazepam (0, 3, 6 and 12 mg/kg, p.o.) on the rectal temperature of male mice are shown on the stress-induced hyperthermia (SIH) paradigm (top) and on isolated male mice. In the top part of the figure the temperature of mouse 1 and mouse 10 (N=8 each) is depicted. * indicates a significant difference from the corresponding vehicle. In the box on top of this figure it is indicated whether the drug exerts an anxiolytic activity. In the bottom figure the effect of diazepam on the rectal temperature of isolated male mice (N=8/dose) is shown. All injections were made 60 minutes before rectal temperature measurements.

The difference between the mean rectal temperature of mouse 1 vs. mouse 10 is approx. 1.6°C Diazepam dose-dependently decreases the basal temperature (mouse nr. 1) by approx. 0.8°C at 12 mg/kg orally. However, diazepam reduced the enhanced temperature of mouse nr. 10 far more (approx. 2.7°C thereby illustrating its potent anxiolytic efficacy. For comparative reasons we also measured the rectal temperature of isolated mice (fig. 4 bottom). Diazepam here also reduced body temperature, indicating that the decrease in basal body temperature in SIH after diazepam occurs independent of the housing condition (isolated versus grouped).

Table 1: Affinities (Ki in nM) of serotonergic drugs for the various 5-HT receptor sites and other significant binding affinities. *Significant defined as less than a factor 100 difference from the highest affinity. >means > 10,000 nM.

Receptor	5-HT						Uptake	other significant affinities (*)	agonist (Ag) or antagonist (Ant)
	1A	1B	1C	1D	2	3			
Serotonin	3.1	3.4	3.1	2.8	2.500	4.3	930		**Ag** (by definition)
8-OH-DPAT	2.8	1.800	7.800	930	>	2.95	780		Ag (by definition)
Buspirone	15	3.000	4.800	>	1.000	>	>	D_2; Σ	Ag (1A)
Ipsapirone	5.5	3.500	>	>	2.700	>	>	D_2; α	Ag (1A)
Flesinoxan	1.7	810	>	160	4.500	>	>	D_2	Ag (1A)
Gepirone	26	8.500	>	>	3.800	>	>	D_2	Ag (1A)
NAN-190	1.3	620	630	790	220	>	2100	α_1; D_2; Σ	Ant (1A, α_1)
WAY 100, 139	10	8.900	5.300	1.000	1.800	>	>	D2;α 1	Ant (1A)
(S) - (-) pindolol	210	400	>	8.700	>	>	9.100	β	Ant (1A, 1B, β)
(±) propranolol	140	540	1.100	>	>	4.000	1.800	β	Ant (1A, 1B, β)
TFMPP	200	49	13	690	780	2.100	1.100		Ag (1C, 1B)
mCPP	210	79	29	1.100	140	6.2	270	α_1, α_2	Ag (1B, 1C)
5-MeODMT	8.5	85	20	39	2.600	5.600	7		Ag (1A, 1B, 1C, 1D)
RU 24969	8.7	5.9	48	42	1.700	3.800	200		Ag (1A, 1B, 1C, 1D)
Eltoprazine	40	52	81	390	1.700	25	>	β; α_1	Ag (1A), 1B, Ant (1C)
Cyanopindolol	5.9	17	>	410	>	>	-	β	Ant (1A, 1B, β)
DOI	6.900	2.100	6.5	7.200	210	>	>		Ant (1C, 2)
Ritanserin	830	1.700	0.55	410	3.2	7.200	1.15	D_2;H_2;α_1	Ant (1C, 2)
Ketanserin	>1.000	>	110	2.200	1.7	>	>	α_1, D_2	Ant (2)
Sumatriptan	250	160	8.1	68	>	>	>		Ag (1A, 1B, 1D)
Ontansetron	>	3.700	5	>	>	1.6	>		Ant (3)
Granisetron	>	>1.000	>	>	>	0.48	>		Ant (3)
Zacopride	>	>	2.600	>	3.600	0.53	>		Ant (3)
ICS 205, 930	>	>	>	>	>	1.6	690		Ant (3)
2 methyl-5-HT	1.7	810	540	1.800	>	36	>		Ag (3)
Quipazine	2.3	620	72	1.800	1.1	0.19	49		Ant (3), Ag (1C)
Phenylbiguanide	>	>	>	>	>	8.7	>		Ag (3)
3,4-DiCIPB	>	>	1.1	>	1.100	1.1	830		Ag (3)
Fluvoxamine	>	>	>	>	1.400	>	5.1		Ant (upt.)
Zimelidine	>	>	4.9	>	3.600	3.200	150	α_1, Σ	Ant (upt.)
Fluoxetine	>	>	830	>	2.300	4.200	11.7	M_1, Σ	Ant (upt.)
Chlorimipramine	>	>	130	>	74	850	2.8	Σ, α_1, D_2 musc, H_1	Ant (upt.)

3,4-diCIPB= 3,4 dichlorophenylbiguanide. NAN-190= (1-(2-methoxyphenyl) -4-(4-(2-phthalamido)butyl) piperazine HBr; WAY 100, 139= (N-tert-butyl 3-4-(2-methoxyphenyl) piperazin -1-yl-2-phenylpropranamide dihydrochoride; : TFMPP=meta-trifluoromethylphenylpiperazine; mCPP=m-chlorophenylpiperazine; 5-MeODMT=5-methoxy-N, N-dimethyltryptamine;

RU24696 = 5- methoxy-3-(1, 2, 3, 6-tetrahydropyridin-4-yl) -1H indole succinate; DOI =(±)-1-1 (2,5-Dimethoxy-4 idophenyl-2 aminopropane hydrochloride; ICS205, 930= 1 aH, 3a, 5aH-tropan-3yl1H-indole-3-carboxylate.

Table 2: Serotonergic Syndrome in the Rat. LLR=Lower Lip Retraction, WDS=Wet Dog Shake, FBP=Flat Body Posture, HDW=Head Weaving, FPT= Fore Paw Treadin, PC= Purposeless Chewing

	5-HT mechanism of action Parameters											
Drugs	1A	1B	1C	2	3	uptake	LLR	WDS	FBP	HDW	FPT	PC
5-MeODMT	+	+	+			-	+	+	++	+	++	0
8-OH-DPAT	+						++	0	+	+	+	0
Flesinoxan	+						++	0	++	+	+	0
Buspirone	+						++	0	++	0	0	0
Ispapirone	+						+	0	+	0	0	0
WAY100, 135	-						0	0	0	0	0	0
RU 24969	+	+	+				++	0	++	++	0	?
Eltoprazine	+	+	-		+		++	0	++	0	0	?
TFMPP		+	+				0	+	+	+	0	++
mCPP		+	+		+		0	+	++	0	0	++
Quipazine			+	+	-		0	++	++	+	+	+
DOI			+	+			0	++	+	+	+	+
Ketanserine			-	-			0	0	0	0	0	0
Ritanserine			-	-			0	0	0	0	0	0
Ondansetron					-		0	0	0	0	0	0
Fluvoxamine						-	0	0	0	0	0	0
Fenfluramine (release)							0	0	0	+	++	+

-+ = agonist; -= antagonist

0= no effect; +=moderate
++ = strong

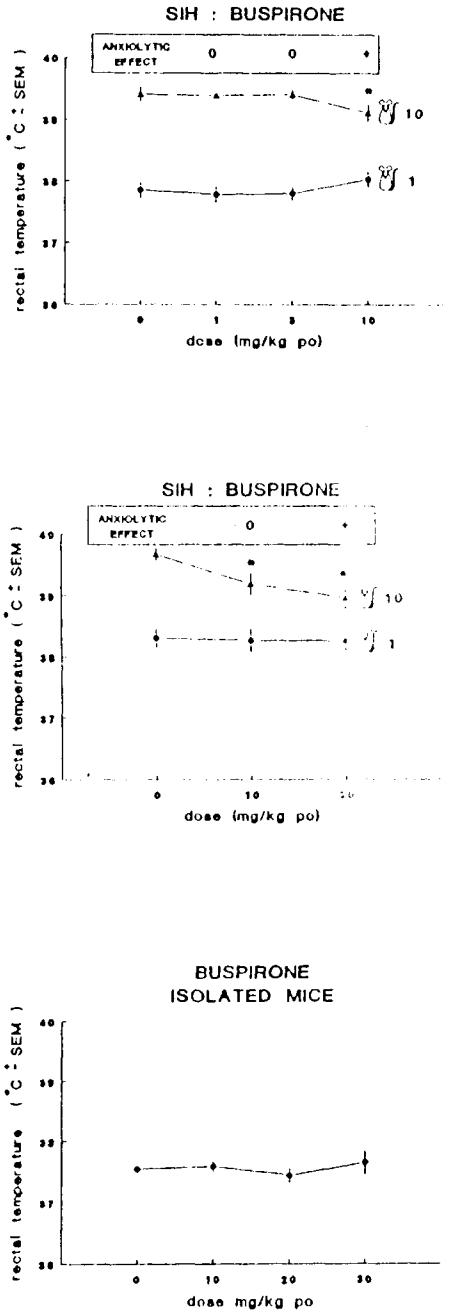

Fig. 5 See legend fig. 4. Buspirone has been tested in two dose ranges in SIH; one from 0 to 10mg/kg po (top left), the other from 0 to 20 mg/kg po (top right). The effects of buspirone (0-30 mg/kg, p.o.) on isolated mice is shown in the bottom figue.

5-HT1A-receptor agonists are also active in this paradigm (fig. 5). As an example buspirone is given, a partial receptor agonist. At relative high doses (10, but particularly 20 mg/kg) an anxiolytic activity is seen, whereas no effect on basal temperature is noted. Similarly, when measured in isolated mice (fig. 5 bottom) no effect of buspirone was found on temperature. Comparable effects were found after other 5-HT1A receptor agonists like ipsapirone or flesinoxan. This is a remarkable phenomenon because several reports describe 5-HT1A receptor agonists as potent hypothermic agents both in rats and mice (36), although Lecci et al. (18, 49) did also not find effects of 8-OH-DPAT and buspirone on the basal temperature in mice measured in an SIH-paradigm. Specific 5-HT reuptake blockers like chlorimipramine and fluvoxamine and 5-HT3 receptor antagonists (e.g. ondansetron) have no effect on this measure.

Besides measurement of effects of drugs on basal (core) temperature, this test measures also aspects of anxiety or stress-related phenomena. Therefore, it is a very interesting model which adds considerable to our armentarium for detection of new anxiolytic agents.

BEHAVIOURAL STUDIES

Behaviour can be used in two ways. First, as an "in vivo bioassay" in which (one aspect of) behaviour is used as a parameter of activation of a certain neuronal mechanism. In this chapter the induction of serotonergic behaviour (the serotonin syndrome) is used as such.

Second, behaviour can be used functionally as part of a complex representing some form of animal model for a human psychiatric disease. Such therapeutic models are repkesented here in animal models of anxiety (ultrasonic pupvocalisation), depression (forced swimming) and aggression (resident-intruder).

The "Serotonin Syndrome"

First, a simple model is illustrated, viz. the direct observable effects of 5-HT ligands on behavioural signs and reflexes. Administration of a variety of 5-HT agonists may produce a series of behaviours known as the 5-HT behavioural syndrome or "serotonin syndrome" (sse 93). These "motor" effects can be easily observed and table 2 shows for a number of 5-HT ligands how they induce a selected number of effects in rats.

5-HT1A receptor activation specifically induces lower lip retraction (7, 58), whereas also flat body posture seems to be related to 5-HT1A receptor activation. Lower lip retraction (LLR) induced by 8-OH-DPAT can be antagonized by pindolol, spiperone, spiroxatrine and NAN-190, but not by metoprolol, butoxamine and haloperidol, strongly indicating that LLR is a very specific 5-HT1A receptor mediated response (8).

Wet dog shaking seems to be related to activation of 5-HT2(A) and (although to a lesser extent) 5-HT1C(2C) receptors. Leadweaving and forepaw treading (piano playing) are less clearly related to activation of a certain 5-HT receptor subtype. Activation of 5-HT1D receptors is as yet an unresolved issue. Although 5-HT1D receptors do occur in rodents, no data are available on their activation, althugh recently scratiching was described as a peripheral 5-HT1D receptor mediated effect (6). No clear behavioural pattern has been observed after activation of 5-HT3 receptors, but this may be due to the poor penetration of the brain by the presently available 5-HT3 receptor agonists (2Me-5HT; 3Cl-PB; 3, 4, diCl-PB). No serotonergic responses have been observed after 5-HT3 receptor antagonist administration.

The agonist-induced behaviours are very suitable to describe the behavioural consequences of selective activation of certain 5-HT receptor subtypes and can be described as behavioural biossays, but do not give information on the role of these different subtypes in various behaviours or behavioural disturbances.

Therefore, animal modeğls representative for a human disorder have to be used and the effects of various serotonergic agents must be determined, possibly leading to a putative hypothesis about the involvement of 5-HT receptor subtypes in the underlying pathological condition. An extensive number of animal models is in use to determine e.g. the anxiolytic, antidepressant and antipsychotic effects of drugs. Moreover the effects of drugs on feeding, sexual, aggressive and other behaviours can be assessed.

THERAPEUTIC MODELS

Animal therapeutic models try to simulate the corresponding human disease. In the case of psychiatric diseases this simulation appears an extremely difficult enterprise, largely due to the fact that a psychiatric disease itself is often subject to dispute about its content, boundaries and aetiology.

Nevertheless, several attempts have been and will be done in future in order to model (parts of) the disease. Three facets of a model may be taken into account, viz. the predictive validity, the face validity and the construct validity. Predictive validity indicates that performance in the test predicts performance in the disease; face validity means that there are phenomenological similarities between model and disease; and construct validity implies a sound theoretical rationale for the model (95).

Predictive validity comprises a number of features which determine the belief in the model, including differentiation between false positive and false negative responses, the sensitivity of the model for treatments that make the clinical condition worse (e.g. in the case of anxiety; anxiogenic drugs); and response to acute or chronic treatment. It is practically never possible to fulgil all these criteria, but the model should not deliver too many discrepancies with the human disorder.

The end result is often an animal model of a certain psychiatric disease with a lot of inherent problems. In the case of e.g. anxiety models, the existing models tend to be oversensitive to benzodiazepines, the standard anxiolytics of the last three decades. However, almost all of these models appeared to be relatively insensitive to non-BDZ anxiolytic drugs, like the 5-HT1A receptor agonists. It can be concluded therefore that the classical anxiolytic models have only limited predictive validity, whereas face and construct validity also remain rather equivocal.

Newer animal models better representing clinical anxiety disorders are therefore urgently needed. Similar pictures hold for the fields of depression, psychosis and others.

It is tried to give examples of animal models representing (part of) certain psychiatric disorders. In these models (ultrasonic pup vocalization in rats; forced swim in rats; resident-intruder aggression in rats) the effects of serotonergic ligands is illustrated and compared to the more classical drugs.

Ultrasonic Pupvocalisation

In the present paper one example of an animal model of anxiety is chosen, viz. an animal model which putatively reflects "anxiety" and consequently allows to detect anxiolytic activity of drugs. This model, ultrasonic distress vocalisations in infant rats, has been shown to detect anxiolytic activity of putative anxiolytics, including benzodiazepines (27, 28, 43), 5-HT1A receptor agonists (59), 5-HT reuptake blockers (59, 96) and some kynurenic acid derivatives (45, 99). Some putative anxiogenic drugs enhance calling (30, 43), whereas a number of other psychotropic agents devoid of anxiolytic properties are inactive (28).

Before postnatal day 18 infant rats emit bouts of ultrasonic calls when separated from the mother, which reacts with searching and retrieving the pup. Besides by mere separation, calling is also enhanced by stressful stimulation like exposure to temperatures below those in the nest (2, 66), to tactile stimulation (75). Stimuli from the mother and /or littermates have a queting effects (38). On these grounds infant rat ultrasonic vocalisations can be taken to reflect a state of distress.

In this test, we separated pups of 9-11 days of age from their mother and littermates and after i.p. injection, their calling was measured under low stress (37°C-warm plate) and high stress (18°C-cold plate) conditions during a 5 min. test (cf. 59, 88).

Various serotonergic agents have been tested in this paradigm. A typical example of the effects of the standard reference 5-HT1A receptor agonist 8-OH-DPAT is shown in figure 6.

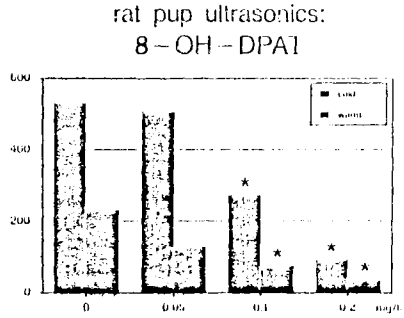

Fig. 6. The number of ultrasonic calls of young rat pups (9 days old) in a 5 minute teste on a cold plate (18°C) or on a warm plate (37°C) as a function of the dose of 8-OH-DPAT (mg/kg, i.p.) given 30 minutes before the test. Data are portrayed as the mean (+SEM) number of calls. *:$p<0.05$ significant diffence from vehicle treatment (0 mg/kg).

8-OH-DPAT dose-dependently reduces ultrasonic calling under both low and high-stress conditions. An overview of the effects of various other 5-HT agents is given in table 3.

As can be seen 5-HT1A receptor agonists (8-OH-DPAT, ipsapirone, buspirone, flesinoxan) reduce ultrasonic calling under both temperature conditions. (±)- Pindolol and (±)-propanolol, both reduce ultrasonics on the warm, but not on the cold plate. This is somewhat unexpected, as both compounds are putative 5-HT1 receptor antagonists (besides their strong ?-adrenergic antagonistic activity). However, there is evidence from drug discrimination studies (101, 102) that both compounds exert (partial) agonistic 5-HT1 activity, as they may substitute for the cue of some 5-HT1 agonists, like TFMPP (33) and eltoprazine (102).

Mixed 5-HT1 agonists (RU 24969, eltoprazine, TFMPP) have no effects on calling on the warm plate, but decrease it on the cold plate. At the lowest dose tested (0.3 mg/kg) RU 24969 enhanced calling on the cold plate, but inhibited it at higher doses. Because these drugs share agonistic activity at the 5-HT1B and 5-HT1A receptor, but not at the 5-HT1C receptor (eltoprazine is a 5-HT1C receptor antagonist) and based on the potency range for RU 24969 (5-HT1A ?5-HT1B>5-HT1C), for eltoprazine (5-HT1A ?5-HT1B>5-HT1C) and TFMPP (5-HT1C>5-HT1B>5-HT1A) it could be concluded that this profile (not active at warm plate, inhibitory at cold plate) is due to activation of the 5-HT1B receptor. Aparently, the activation of the 5-HT1A receptor (which is quite marked e.g. in RU 24969) is masked by the simultaneous activation of the 5-HT1B receptor.

Activation of 5-HT1C and 5-HT2 receptors by DOI leads to inhibition of calling under both contitions. We find ketanserin and ritanserin equally effective in enhancing calling, which agrees well with the nearly equal affinities for 5-HT2 receptors of both drugs (table 1). The affinity of ritanserin for 5-HT1C receptors is considerably higher than that of ketanserin, making the involvement of this receptor less likely. Winslow and Insel (97, 98) studied the interaction of ritanserin with DOI and m-CPP on ultrasonic calling. Ritanserin counteracted the suppressing influence of DOI but potentiated that of m-CPP. Since both DOI and m-CPP are non-selective agonists at 5-HT2 and 5-HT1C

Table 3: Ultrasonic vocalization of rat pups (9-11 days old) on two stress conditions, a warm (37°C) and a cold (18°C) plate. The specificity of effect was measured on an inclined screen, where pups were required to turn against a negative geotaxis. ++ = large distance between side effects and effects on vocalisation; + = moderate distance; – = no distance between side effects and effects on vocalisation; 0=not applicable.

Drugs	Warm plate 37°C	Cold plate 18° C	Specificity of effecty
8-OH-DPAT	↓↓↓	↓↓↓	++
Ipsapirone	↓↓	↓↓	++
Buspirone	↓↓	↓↓↓	+
Flesinoxan	↓↓	↓↓↓	++
(±) Pindolol	↓	0	+
(±) Propranolol	↓	0	++
WAY 100, 169	0	0	0
RU 24696	0	↑↓↓	+
Eltoprazine	0	↓↓	++
TFMPP	0	↓	++
mCPP	0	↓	+
DOI	↓↓	↓↓	++
Ritanserin	↑	↑↑	+
Ketanserin	↑↑	↓↓	+
Ondansetron	0	0	0
Fluvoxamine	↓	↓↓	++
Zimelidine	0	↓↓	+
Fluoxetine	↓↓	↓↓	+
Tianeptine	↓↓	↓↓	–

↓= decrease; ↑= increase; 0= no effect; - =not applicable

Table 4: Effects of serotonergic ligands in the forced swimming model in rats. The activity of drugs is given as ED8", the dose needed to lower the immobility by 8 seconds.

Drug	Nature of interaction with the 5-HT system	ED8" (mg/kg s.c.)
8-OH-DPAT	1A agonist	0.025
flesinoxan	1A agonist	0.2
ipsapirone	1A agonist	6.8
buspirone	1A agonist	29
5-Me-ODMT	1A/1C agonist	2
TFMPP	1C/1B agonist	>3
eltoprazine	1A/1B agonist	1.5
DOI	1C/2 agonist	>1
(±) propranolol	1A antagonist/1B agonist	>10
methiotepin	1C/2 antagionist	8.3
ritanserine	1C/2 antagionist	11.5
mianserine	1C/2 antagionist	1.7
clozapine	1C/2 antagionist	1.5
methysergide	1/2 antagonist	>3
ketanserine	2 antagonist	>10
ondansetron	3 antagonist	>10
fluvoxamine	reuptake blockade	>30 (i.p.)
fluoxetine	reuptake blockade	>30 (i.p.)
chlorimipramine	reuptake blockade	> 30 i.p.
paroxetine	reuptake blockade	>30 i.p.
trazodone	reuptake blockade, agonist (1A, 1C)	0.4
tianeptine	reuptake stimulant	>30 (i.p.)

receptors (table 1) and were studied at one dose level only, this evidence is tenuous.

Ondansetron, a specific 5-HT3 receptor antagonist is devoid of activity under both conditions over a wide dose range.

5-HT reuptake blockers (fluvoxamine, zimelidine, fluoxetine) mildly decrease calling on the warm plate and moderately decrease calling on the cold plate. Tianeptine, a 5-HT reuptake stimulant strongly decreases calling under both conditions but this is presumably caused by nonspecific effects.

Considering the overall profile of the different 5-HT ligands, the ultrasonic vocalisation test seems to differentiate the involvement of the various 5-HT receptors in this anxiety model. The following tentative conclusions can be drawn.

Activation of 5-HT1A receptors strongly decreases calling under low and high stress conditions, whereas activation of 5-HT1B receptors seems to decrease calling only under high stress conditions. The role of 5-HT1B receptors seems to decrease calling only under high stress conditions. The role of 5-HT1C and 5-HT2 receptors is intermingled and difficult to attribute to either one of them. Activation of these receptors moderately decreases calling under both stress-conditions, whereas blockade of these receptor anhances calling. Blockade of 5-HT3 receptors has no infuence on calling. In the absence of specific 5-HT3 receptor agonists no definite conclusions can be drawn about the role of these receptors in distress calling. 5-HT reuptake blockers have mild to moderate decreasing effects on the warm plate and moderate decreasing effects on the cold plate. As these compounds enhance 5-HT levels in the synaptic cleft and thus act as indirect agonists at the various 5-HT receptors, it is difficult to judge via which receptor(s) these effects are mediated. However, the vocalization test clearly can be of help to differentiate the various serotonergic agents with regard to their mechanism of action.

FORCED SWIMMING TEST

In this procedure, originally described by Porsolt and coworkers (79), rats or mice are forced to swim in a clinder filled with water and where escape is impossible. Untreated animals try to escape vigourously but after some time (minutes) seem to surrender, reflected in the long periods of immobility (floating), now and then interspersed by small movements. In such an experiment immobility is measured during a number of minutes (in our tes 6). An antidepressant effect is shown by a decrease in the time spent on immobility. The immobility parameter in this model appears to be sensitive (decreased) to electroshocks (ECS) and most antidepressants, including tricylics, MAO-inhibitors and also newer antidepressants, both after acute and subchronic administration. Benzodiazepines are not active in this test. The model seems to have good predictive, face and construct validity (14, 94).

In our procedure (89) rats are placed for 15 minutes in a cilinder (diameter 20 cm) with water of 25°C (height 15 cm). Immediately hereafter animals are removed and injected with drug. The next day the animals are treated again with the same drug (and same dose) 23.5 hours after the first treatment; 30 mins later the animals are again placed into the cilinder ant dhe subsequent 6 minutes the duration of immobility is measured using a computerized videotrack system. Table 4 shows the effects of various serotonergic ligands in this forced swimming model.

8-OH-DPAT is a very potent drug in this forced swim-model (fig. 8) and reduces immobility already at 0.03 mg/kg s.c. This holds also for other 5-HT1A receptor agonists like buspirone, ipsapirone and flesinoxan (table 4). Mixed 5-HT agonists are active in the forced swimming tes (5-Me-OMT, eltoprazine) at least when they eert a 5-HT1a-agonistic property.

5-HT1B/1C receptor agonists (TFMPP, DOI) are inactive, like the 5-HT1A antagonist/5-HT1B agonist (±)- propranolol (which is also a potent ?-noradrenergic blocker). Mixed 5-HT1C/2 receptor antagonists (methiotepin, ritanserine, clozapine, mianserin) are active in the forced swimtest, suggesting that in particular the 5-HT1C antagonism is important, because ketanserine (primarily a 5-HT2 receptor antagonist) is inactive. Ondansetron, a specific 5-HT3 receptor antagonist is inactive, Specific 5-HT reupake blockers like fluvoxamine, fluoxetine, chlorimipramine and paroxetine are not active in the swimtest. Trazodone, a settled antidepressant is also active in the forced swimtest. Its mechanism of action is complex, involving 5-HT reuptake blockade, 5-HT1A/1C agonism and some releasing properties, which makes its activity less easy to ascribe to a single serotonergic mechanism.

These data are generally in agreement with those in the literature (80), although the effect of SSRIs is not always found. This model, and related behavioural despair models, therefore seem to have

good predictive validity. Whether the model can be used to pick-up all putative antidepressants is not clear. At least it is in our hands not sensitive towards SSRIs, whereas the 5-HT1C receptor antagonists, which are predicted in the model to be antidepressants but not yet clincically proven, could be false positivies.

Resident-Intruder Offensive Agression

Rats are socially living animals (3, 87) and male rats defend territories (3) against unfamiliar intruders. This territorial behaviour can be easily demonstrated under semi-natural laboratory conditions using domesticated and inbred strains of rats (11, 50, 67). Introduction of an unfamiliair male in a territory evokes a complete agonistic repertoire, similar to that under natural circumstances (3, 87). The occurence of a rich, natural behavioural repertoire is used in the pharmacological study of serotonergic agents on this kind of offensive aggression. By recording a wide variety of behaviours it is possible to detect very specific changes and gain information about the mechanism of action of drugs (56, 57, 73, 74). Details of the methodology are elsewhere provided (68, 74).

Briefly, male rats (residents) living together with a female in a large territorial cage, were confronted during 10 mins with a strange male intruder. Behaviour was recorded using ethograms as described before (68).

The results for a number of serotonergic ligands are schematically shown in table 5.

Buspirone, a 5-HT1A receptor agonist dose-dependently reduces aggression, but simultaneously social interest and exploration. Because inactivity (mainly comprising sitting and lying) is concomitantly strongly enhanced, the antiaggressive profile of buspirone is characterized as nonspecific , presumably caused by a strong sedatory component in the drug due to its strong dopamine (D2)-antagonistic properties. However, other 5-HT1A receptor agonists (8-OH-DPAT, ipsapirone, flesinoxan) share this non-specific decrease in aggression (60, 61), suggesting that activation of 5-HT1A receptors modulates offensive aggression in a non-specific way.

Mixed 5-HT1 receptor agonists like TFMPP (1C, 1B, 1A agonist), RU 24696 (1A, 1B, 1C agonist) and eltoprazine (1A, 1B, agonist; 1C antagonist) specifically decrease aggression, because they do not simultaneously decrease social interest and exploration and enhance inactivity. Considerable effort has been made to find out whether one specific 5-HT receptor is involved in the specific modulation of offensive aggression, pointing to the 5-HT1B receptor as the primary candidate.

Fluvoxamine, a specific 5-HT reuptake blocker only reduces aggression at a relatively high dose, where already non-specific affects are noted (social interest and inactivity), suggesting that such a broad mechanism, enhancing 5-HT neurotransmission does not lead to specific decreases in offensive aggression.

Extensive studies using a variety of aggression paradigms has supported the hypothesis that the 5-HT1B receptor in rodents fulfills a specific modulatory role in offensive aggression. Table 5 illustrates the various 5-HT principles in a variety of animal models representing offensive and defensive aggression.

5-HT1A Receptors

It can be seen that 5-HT1A receptor agonists (8-OH-DPAT, buspirone, gepirone, flesinoxan) have anti-aggressive activities in different paradigms for offensive aggression (isolation-induced aggression in mice, resident-intruder and maternal aggression in rats, but these studies also showed the behavioural nonspecificity of these anti-aggressive effects (69, 70, 71). Moreover, in some paradigms (hypothalamic-induced aggression in rats, muricide in rats) 5-HT receptor agonists have no anti-aggressive activity (69, 71). This may indicate that 5-HT1A receptor activation in some situations induces behavioural disturbances leading to an antiagressive action, but that there is no direct modulation of aggression.

A remarkable observation is that propranolol, a proposed 5-HT1A antagonist and a potent β-noradrenergic blocker, has anti-aggressive activity in some models, although not in others. There is evidence however, that propranolol has also a 5-HT1B agonistic activity (102) which may explain propranolol's relatively specific anti-aggressive activity.

5-HT1B Receptors

All available data (69, 70, 71, 73) point to the highly specific role for 5-HT1B agonists in decreasing offensive aggression. Although no specific 5-HT1B agonists are present, but only mixed 5-HT1 agonists, the available agents (eltoprazine, RU 24969, TFMPP) indicate a role for

Table 5. Summary of the effects of serotonergic drugs on several aggression paradigms.

		Aggression paradigm							
		IIA	SI	RI	MA	BI	MU	FID	DI
5-HT1A	Agonists	0/↓	0/↓	↓	↓	0	0	0	-
	Antagonists	0	0	+	0	+?	0	0	-
5-HT1B	Agonists	+	+	+	+	+	+	0	0
	Agonists	0	0	+	0	+?	0	0	-
5-HT1C	Antagonists	0	0	↓	↓	↓	↓	-	-
	Agonists	0	0	-	0	0	0	0	-
5-HT2	Agonists	0	-	↓	↓	↓	-	-	-
	Antagonists	0	0	-	-	0	0	0	-
5-HT3	Agonists	-	-	-	-	-	-	-	-
	Antagonists	0	-	0	0	0	-	-	-

IIA= Isolation-induced aggression (mouse); SI= social interaction (mouse); RI= resident-intruder (rat); MA= maternal aggression (rat); BI=brain-stimulation induced (rat); MU= muricide (rat); FID= foot-shock-induced defense (mouse); DI= defense intruder (rat).
+= Specific reduction; ↓= non-specific reduction; 0=no effect; –= not investigated; ?= ambiguous.

Table 6 Effects of various compounds in generalisation and antagonism studies in flesinoxan (0.3 or 0.5 mg/kg) and eltoprazine (0.5 mg/kg) versus saline trained rats. Generalisation: >80% on correct lever; Partial generalisation between 20 and 80% correct lever pressing

	Training Drugs	
	Flesinoxan	Eltoprazine
Generalisation	8-OH-DPAT Ispapirone Yohimbine Gepirone	8-OH-DPAT TFMPP Fluprazine RU 24969 (±) Pindolol (±) Propranolol
Partial Generalisation	Buspirone Idazoxan	Buspirone Flesinoxan mCPP Mesulergine Idazoxan Fluvoxamine
No Generalisation	Eltoprazine TFMPP RU 24969 Fluvoxamine Methysergide (±) Pindolol Quipazine Ketanserin Mesulergine DIO	ICS 205, 930 Ketanserin DOI Timolol NAN-190
Antagonism	(±) Pindolol HAH190 (partial) (±) Propranolol	
No Antagonism	Idazoxan Methysergide ICS 205,930 Katenserin Mesulergine	ICS 205,930 (±) Pindolol (±) Propranolol Mesulergine Katenserin Mehtysergide Timolol NAN-190 Idazoxan

the 5-HT1B receptor in aggression, at least in rodents. Whether the 5-HT1D receptor fulfills a comparable role in non-rodent species with regard to aggression awaits the availability of specific 5-HT1D ligands.

5-HT1C/1D Receptors

The role of the 5-HT1C receptor in aggression is not clear. The available agonist data (DOI: a 5-HT1C/2 agonist) reveals a nonspecific inhibition of aggression, whereas most work using antagonists (ritanserin; ketanserin) shows no antiaggressive effects. This may suggest that the 5-HT1C receptor is not involved in the modulation of offensive aggression (70, 71). Unfortunately, no specific 5-HT1D ligands are available (sumatriptan has also affinity for the 5-HT1A and 5-HT1B receptors, but more importantly does not cross the blood-brain barrier) and when such compounds become available, they have to be tested in non-rodent species because of the absence of the 5-HT1D receptor in rats and mice.

5-HT2/5-HT3 Receptors

For 5-HT2 an identical story holds as for the 5-HT1C receptor. The 5-HT3 receptor, at least as far as modulated via 5-HT3 antagonists, seems not to be involved in aggressive behaviour (70, 71).

DRUG DISCRIMINATION

Another approach chosen is drug discrimination (DD). DD has not been developed as a model and cannot simply be fitted into classifications of animal models like empirical, physiological or homologous models (cf. 85 for an extensive discussion).

For the present purpose we restrict ourselves to DD as a means of analysing the central mode of action of drugs. Drug discrimination procedures seem a very sensitive "in vivo" bioassay to study the mechanism via which a certain compound interacts with a receptor system and through which it produces its specific stimulus effects (cues).

In the drug discrimination procedure, experimental subjects (ranging from rats, pigeons, monkeys to humans) are trained to perform one response in the presence of a selected drug and a different response when no drug (vehgicle) or a different drug is present. Thus, the correct choice depends on the injection condition. Commonly, this consists, of pressing either one of two lvers (rats) or pecking at keys (pigeons) in an operant chamber (Skinnerbox). After having reached stable choice levels (criterion >80% on cerrect lever) animals are subjected to generalization (substitution) and antagonism tests.

Drug discrimination techniques have proven to be extremely useful in classifying the drugs according to their mechanism of action. Also in the serotonergic field a considerable number of studies has been performed, including hallucinogenics like LSD, DOM and DOI (31), 5-HT1A receptor agonists (4) and mixed 5-HT1A receptor agonists like RU 24969 (29) and TFMPP (24, 32, 33).

For the present purpose studies with the 5-HT1A receptor agonists flesinoxan and the 5-HT1A/1B receptor agonist eltroprazine will be shown, using DD procedures in rats and pigeons. Rats are trained to discriminate flesinoxan (0.3 or 0.5 mg/kg i.p.) from saline or eltoprazine (0.5 mg/kg i.p.) from saline. Table 6 illustrates the generalization and antagonism studies in rats trained with flesinoxan and eltoprazine. These studies are based on data gathered by Ybema et al. (100, 101, 102, 103).

Flesinoxan can easily be trained in rats to function as a discriminative stimulus and various 5-HT1A receptor agonists generalize completely (8-OH-DPAT, ipsapirone, gepirone) or partly (buspirone) to the flesinoxan-cue. Remarkable, two α 2-noradrenergic receptor antagonists (yolinbine and idazoxan-cue. It could be shown (103) that the α 2-noradrenergic component does not contribute to it. Various serotonergic ligands, including 5-HT1B,1C receptor agnoists, 5-HT2 and 5-HT3 receptor antagoinists and various non-serotonergic agents e.g. dopaminergic and adrenergic agents, did not substitute for the flesinoxan cue.

The flesinoxan cue could be antagonized by the 5-HT1A receptor antagonists (±) pindolol and (±) propranolol, but only partly by the putative 5-HT1A receptor antagonist NAN-190. The lack of antagonism by various other compounds, including 5-HT2 and 5-HT3 receptor antagonists underlines the specific character of the flesinoxan-cue in rats: a 5-HT1A receptor mediated cue.

Eltoprazine can also be trained easily in rats to act as a discriminative stimulus and creates a cue with different stimulus qualities than that of flesinoxan (table 7).

8-OH-DPAT, TFMPP, fluprazine, RU 24969, (±) pindolol and (±) propranolol completely substitute for eltoprazine, whereas partial substitution is obtained with buspirone, flesinoxan,

mCPP, mesulergine, idazoxan and fluvoxamine. This indicates that the stimulus effects of eltoprazine are complex and at least involve the 5-HT1A and probably the 5-HT1B receptor. Moreover, it can be concluded that (±) pindolol and (±) propranolol act as (partial) 5-HT1 receptor agonists because they completely substitute for the eltoprazine cue. Since these agents behave like 5-HT1A receptor antagonists in the flesinoxan cue, it can be argued that the 5-HT1B agonistic properties of pindolol and propranolol mediate the generalization to eltoprazine. The eltoprazine cue did neither generalize to, nor was antagonized by 5-HT2 receptor agonists/antagonists and 5-HT3 receptor antagonists.

This paradigm in rats therefore clearly differentiates between a full 5-HT1A receptor agonist (flesinoxan) and a (partial) mixed 5-HT1A/1B receptor agonist (eltoprazine). In this case the behavioural paradigm underlines very elegantly the in vitro data, as e.g. shown in the affinity table (table 1) and elsewhere (81, 82).

However, the rat may not always be the best choice of animal model for extrapolating the data to the human situation. It is known that rats possess 5-HT1A and 5-HT1B receptors (besides all the other 5-HT receptors) whereas in the human the 5-HT1D receptor appears to fulgil the role of the 5-HT1B receptor (40, 90, 91). Therefore, it would be informative to perform drug-discrimination studies in a species which, like humans, has 5-HT1D receptors and lacks 5-HT1B receptors. The pigeon has been chosen by us for this purpose (cf. 91).

Flesinoxan (0.25 mg/kg p.o.) and eltoprazine (5 mg/kg p.o.) were trained versus vehicle in pigeons. Both drugs rapidly gained stimulus control (stimulus control when >80% choice for appropriate drug key). Table 7 shows the effects of generalization studies in flesinoxan-trained pigeons. Only a limited number of drugs has been tested sofar. It was found that eltoprazine, buspirone, ipsapirone and 8-OHDPAT completely subsitituted for the flesinoxan-cue.

Table 7 shows similar data for eltoprazine trained pigeons. Flesinoxan substituted for the eltoprazine cue. Buspirone only partially substited for eltoprazine (max. 50%), whereas ipsapirone did not substitute (approx. 30%) for eltoprazine, even at the high dose of 100 mg/kg. This indicates that the cue of flesinoxan in piegons is purely mediated via the 5-HT1A receptor. However, in the eltoprazine effect, something else than the 5-HT1A receptor must be involved. As this cannot be the 5-HT1B receptor, it may be the 5-HT1D or the 5-HT1C receptor. Further work with various 5-HT receptor agonists and antagonists is needed to unravel the complex stimulus properties of eltoprazine in the pigeon.

This example of drug discrimination of two drugs with different receptor profiles in two different species differing in their receptor "make-up", reveals the subtlety and complexity of the various mechanisms animal organisms are supplied with to optimally interfere with their environment.

CLINICAL APPLICATIONS

Specific 5-HT reuptake inhibitors (SSRIs) are settled antidepressants illustrated by the fact that fluvoxamine, fluoxetine, sertraline, paroxetine and citalopram are in clinical use or in some countries in approval stage (cf. 16, 17, 18). These compounds have been developed in the seventies based on the theory that dysfunction of serotonin neurons is involved in the pathophysiology of depression (21). Several studies have suggested that an abnormality in pre-synaptic serotonergic processes may be present in at least a subgroup of depressed patients. For instance, several studies have shown that the availability of the 5-HT precursor tryptophan is reduced in depressed patients whereas some depressed patients, prone to sucide have low 5-HIAA levels in the CSF, suggestive of a low 5-HT turnover. Moreover, a low 5-HT uptake into blood platelets, decreased number of [3H]-imipramine binding sites and a reduced platelet or whole blood 5-HT and a blunted neuro-endocrine response when the 5-HT system is challenged in depression have been considered as additional evidence for reduced 5-HT functioning in depression.

There is now ample evidence that SSRIs are also clinically effective in various other psychiatric diseases, like panic disorder (PD), obsessive compulsive disorder (OCD), phobias, generalized anxiety disorder, eating disorders (bulimia, anorexia, obesity), addictive behaviours (e.g. alcohol, smoking) and personality disorders (review 17, 18).

Since it is known that 5-HT reuptake carriers or transporters (5-HT) occur both presynaptically in the terminal and on the 5-HT cellbody (somatodendritically) the processes underlying what is ongoing in the 5-HT system after administration of SSRIs have evolved thoroughly.

Acute administration of SSRIs was always assumed to enhance synaptic levels of 5-HT. Recent

Table 7 Effects of various compounds in generalization and antagonism studies in flesinoxan (0.25 mg/kg p.o.) and eltoprazine (5.0 mg/kg p.o.) versus vehicle (tragacanth 1%) trained pigeons. Generalisation: >80% on correct lever; partial generalization between 20 and 80% correct lever pressing

	Training Drugs	
	Flesinoxan	Eltoprazine
Generalisation	Eltoprazine Buspirone Ipsapirone 8-OH-DPAT	Flesinoxan (±) Pindolol (80%) TFMPP
Partial Gneralisation	RU 24969 (60%) Fluvoxamine (40%)	Buspirone (50%) Ipsapirone (35 %) RU 24969 (50%) 8-OH-DPAT (66%) Fluvoxamine (45%)
No Generalisation	TFMPP NAN-190 (±)	m-CPP (±) Propranolol NAN 190
Antagonism	WAY 100135 (partial) NAN 190	(±) Pindolol (partial) NAN 190
No Antagonism		(±) Propranolol

Table 8 Clinical and preclinical evidince for activity of 5-HT ligands in therapeutic areas.

		Clinical (c) or preclinical (pc) evidence for activity in the therapeutic area indicated
5-HT_{1A}	agonist	depression (c), GAD) (c)
	antagonist	anxiety (pc)
$5\text{-HT}_{1D/1B}$	agonist	migraine (c), aggression (pc), OCD (pc?)
	antagonist	depression (pc)
$5\text{-HT}_{1E/F}$	ag/ant	?
$5\text{-HT}_{2(A)}$	ag	?
	ant	depression (pc), anxiety (?pc)
$5\text{-HT}_{2C(1C)}$	agonist	psychosis (?pc)
	antagonist	depression (pc)
5-HT_{3}	agonist	?
	antagonist	anxiety (pc), psychosis (pc), drug abuse (pc), memory (pc)
5-HT_{4}	ag/ant	?
5-HT_{5}	ag/ant	?

microdialysis studies in rats (1, 5) showed that after acute systemic SSRI administration (clomipramine, fluvoxamine) 5-HT was particularly enhanced in the raphe nuclei whereas no or a very limited increase was found at synaptic levels (frontal cortex). This can be explained by the presence of somatodendritic 5-HT1A receptors which upon activation inhibit cell firing and consequently 5-HT release. Therefore only a limited efficacy of the synaptic blockade of the 5-HTT can be expected (fig. 7).

After chronic administration, which is needed to obtain clinically relevant therapeutic effects, the process of 5-HT reuptake blockade is still effectively present. However the somatonderitic 5-HT1A receptors are desensitized and consequently the serotonergic neuron is not (or less) inhibited anymore by the high levels of 5-HT around the cell body. This leads to a normal 5-HT release, with enhanced synaptic levels of 5-HT because the

synaptic 5-HTT is still blocked. There is even some evidence (64) that the symaptic 5-HTT is downregulated which may mean that the process of 5-HT uptake is even further decreased. It is also found that the presynaptic autoreceptor (5-HT1B/1D) is downragulated (13). This may further enhance 5-HT transmission.

The net effect of chronic administarion is therefore (strognly) enhanced 5-HT neurotransmission. Postsynaptically an extensive range of 5-HT receptors is present (5-HT1A,, 5-HT1B/D, 5-HT1E, 5-HT1F, 5-HT2(A), 5-HT2(C), 5-HT3, 5-HT4, 5-HT5) and it is very likely that the differential effects of 5-HT reuptake blockers (depression, anxiety, etc) can be ascribed to the activation of 5-HT on one (or more) of these sepicific 5-HT subtype receptors.

There is abundant evidence that 5-HT1A receptor agonists exert anxiolytic and antidepressant effects. Buspirone is registered for generalized anxiety disorder and is also in development for depression. Several other 5-HT1A receptor agonists are currently in development for anxiety disorders and depression (ipsapirone, gepirone). It is as yet unknown whether these drugs act via the somatodendritic 5-HT1A receptor, via the postsynaptic one or both. It is clear however, thet 5-HT1A receptor agonists have like SSRIs a delayed onset of action (3-4 weeks). Several serotonergic approaches are presently persuaded by different pharmaceutical companies to figure out whether certain receptors are involved in particular diseases. Table 8 gives a schematic overview of what is presently available and subject to clinical or preclinical investigation.

Conclusions

This paper has taken several examples to illustrate how different behavioural approaches can be of help in determining the fuunctional role of the various 5-HT receptor subtypes. The emerging syntehsis is that no single behavioural test by itself provdies all the information necessary to characterize fully the behavioural effects of the drug under investigation. Various behavioural approaches, coming from different methodological backgrounds, are necessary to optimalize the "in vivo" information gathered from the study of animal behaviour.

Used in this sense, behavioural procedures help optimize the prediction towards the application of drugs in the treatment of human disorders.

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