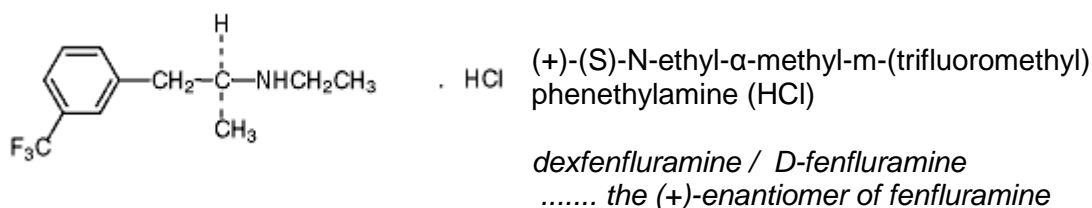


Fenfluramine



A serotonin releasing/uptake-inhibiting agent

Fenfluramine, an amphetamine derivative, devoid of the psychomotor stimulant and abuse potential of d-amphetamine, interacts with 5-hydroxytryptamine (serotonin, 5-HT) transporters to release 5-HT from neurons. Following reports in the late 1970s of the profound influence of fenfluramine on appetite and subsequent reviews of the clinical effects of both fenfluramine and the metabolite norfenfluramine, the long term efficacy of fenfluramine in the treatment of obesity was established. However, anorectic treatment with fenfluramine is associated with the development of cardiac valvulopathy and pulmonary hypertension, including a condition known as cardiac fibrosis which led to the withdrawal of this compound from the U.S. market in 1997. After the US withdrawal of fenfluramine, it was also withdrawn from most other markets around the world. Much of the efficacy of fenfluramine as an anorectic is thought to be derived from activation of the 5-HT_{2C} receptor, whereas interaction with the 5-HT_{2B} receptor is associated with heart valve hypertrophy. Discerning the neurobiology underlying the anorexic action of fenfluramine may facilitate the development of new drugs to prevent and treat obesity.

Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. Circulation. 2000 Dec 5;102(23):2836-41. PubMed PMID: 11104741.

Locke KW, Levesque TR, Nicholson KL, Balster RL. Dexfenfluramine lacks amphetamine-like abuse potential. Prog Neuropsychopharmacol Biol Psychiatry. 1996 Aug;20(6):1019-35. PubMed PMID: 8888108.

Absorption and Metabolism

The absorption half-life of fenfluramine after oral administration to humans is about 1 h, much less than its plasma half-life of 24 h. The pathway for elimination of fenfluramine involves conversion to norfenfluramine, principally in the liver. Norfenfluramine has anorectic and other properties broadly similar to those of fenfluramine and has a plasma half-life about 2 x that of fenfluramine.

The intraperitoneal (i.p.) route of administration is most often used in laboratory studies and provides rapid absorption and bioavailability. Brain levels of fenfluramine and norfenfluramine are substantial and remain high longer than corresponding plasma levels.

In human studies, the plasma level of fenfluramine has been the only direct measure of effective concentrations of drug. Plasma concentrations of about 200 ng/ml (approximately 1 microM) are correlated with the best weight losses and these plasma levels may easily be maintained throughout the 24 h cycle because the plasma half-life is 18-24h. In humans, the concentrations of fenfluramine always exceed those of norfenfluramine despite the slightly longer half-life of the latter.

In rats, a plasma half-life of only about 2 h for fenfluramine, while norfenfluramine has a half-life of at least 12 h. Thus, fenfluramine is the major active compound for only 2-6h after its

administration, and thereafter norfenfluramine predominates. In monkeys and dogs the metabolism of fenfluramine appears to be intermediate between those in rat and man. In mice the half-life of fenfluramine is longer (4.3h) than in rats, and fenfluramine predominates over norfenfluramine at all times, as in man.

Several studies, all in rodents, have described brain levels of fenfluramine and norfenfluramine after a single injection; brain levels are often 10-40x the corresponding plasma levels. The half-life of fenfluramine in rat brain is about 4 h after high doses, but may be shorter after low doses. The half-life of norfenfluramine in brain is at least 24 h. In mice, brain fenfluramine levels exceeded those of norfenfluramine, reflecting the plasma ratio, as in man.

See Review

Rowland NE, Carlton J. Neurobiology of an anorectic drug: fenfluramine. *Prog Neurobiol.* 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Pharmacology

There are several potential mechanisms to explain the acute anorexia after large doses in rodents (e.g. gastric inhibition, glucose and lipid metabolism, brain peptides and serotonin). There are, in contrast, virtually no firm data on the mechanisms of long-term anorexia. The d(+)- and l(-)-enantiomers have distinguishable biological actions but the vast majority of biobehavioural studies have been conducted using the d,l- or racemic mixture.

The effects of *d*-fenfluramine on food intake of rats are considerably greater than those of *l*-fenfluramine, suggesting that the major anorectic action of *d,l*-fenfluramine may be via the *d*-isomer or its metabolite *d*-norfenfluramine. The latter is approximately twice as potent as *d*-fenfluramine in rats, and *d*-fenfluramine is likewise twice as potent as *d,l*-fenfluramine. There is, however, no behavioural evidence that *d,l*-fenfluramine or norfenfluramine have qualitatively different effects upon feeding than either of the *d*-stereoisomers.

The data on the body weight changes seen in free-feeding rats treated daily with *d,l*-fenfluramine or *d*-fenfluramine showed a similar initial weight loss relative to controls, followed by a period of weight gain, usually on a trajectory below that of controls. The effect is not dissimilar from the clinical observations and there is some debate as to whether this constitutes tolerance to weight loss or not.

Weanling rats fed on a 12 h per day feeding schedule with a simultaneous choice between a high protein and a high carbohydrate mixed diet were anorexic to only the carbohydrate diet after fenfluramine. Their intake of the high protein diet was maintained at no-drug levels which indicated that fenfluramine selectively reduced carbohydrate, but spared protein intake. Others also found protein sparing only with a low dose of *d*-fenfluramine, and that higher doses of *d*- or *l*-fenfluramine non-selectively reduced intake. Although later studies, using macronutrient sources, found no evidence for selective protein sparing.

Several studies have reported inhibition of gastrointestinal motility in several species following fenfluramine. Each of the equi-anorectic doses of *d*, *l*, or *d*- and *l*-fenfluramine inhibited motility; phase of propulsion along the intestine although the effect was apparently not statistically reliable for *d*- or *l*- at the doses indicated.

When *d,l*- or *d*-fenfluramine is administered after self-igestion of a chow-meal in various rodent species, there is almost complete inhibition of stomach emptying for several hours. The study suggested that the decreased meal size and/or prolonged inter-meal interval which characterize the action of fenfluramine could be a direct result of gastric slowing.

However, midbrain raphe nucleus lesions abolished the anorectic effect of fenfluramine in deprived rats but had no effect upon its gastric inhibitory action, which suggested that the effect of fenfluramine to decrease meal size may be related to its central 5-HT actions, and be independent of the effect to increase inter-meal interval which may be of gastric origin.

Rats which exhibited tolerance to fenfluramine anorexia in a scheduled feeding paradigm also showed complete and parallel tolerance to its gastric inhibitory effects. In later work, *d*-fenfluramine (2 mg/kg) produced complete inhibition of emptying of the chow-meal, and that tolerance develops to this effect with eight daily injections. Rhesus monkeys exhibited early (volume-related) and late (calorie-related) phases of emptying of liquid meals. Fenfluramine slowed the early phase of emptying, and slowed the later phase in two out of four subjects. In agreement with the animal studies, selective slowing by fenfluramine of emptying of the solid (beef) but not liquid (glucose) components of a mixed meal was found in humans.

The mechanism by which fenfluramine inhibits gastric emptying is unclear although, insofar as *l*-fenfluramine may be less effective than the *d*-enantiomer, a serotonergic mechanism may be implicated. However, neither intestinal nor stomach levels of 5-HT are depleted by fenfluramine.

There is indirect evidence that fenfluramine releases 5-HT from the gut because its administration causes ileal contractions which can be blocked by 5-HT antagonists. However, no decreases in gut content of 5-HT or 5-HIAA have been observed following acute administration of fenfluramine.

Fenfluramine generally enhances carbohydrate metabolism in peripheral tissues. Thus, insulin-dependent glucose uptake is stimulated, glucose tolerance is improved and fasting blood glucose is reduced. Diabetic people and mice showed improved glucose tolerance with fenfluramine. These effects are not secondary to increased insulin release *in vivo*.

However, fenfluramine may enhance insulin receptor sensitivity, or bind directly to insulin receptors. In hepatocytes isolated from food deprived rats, fenfluramine inhibited gluconeogenesis from lactate, pyruvate and alanine.

The facilitatory effects of fenfluramine on glucose uptake are stereospecific, with the *d*-(+)-enantiomer much more potent than the *l*-(-)-enantiomer. These effects appear to be 5-HT-mediated insofar as the *in vitro* stimulation of glucose uptake by fenfluramine is blocked in a dose-dependent fashion by the 5-HT antagonist, methysergide and is enhanced by addition of 5-HT or 5-HTP to the tissue specimen.

Other studies have failed to report a change in basal metabolic rate of humans or rodents treated with fenfluramine. An increase in metabolic rate of rats treated with *d*-fenfluramine was reported, but only during periods of motor activity. Daily injection with *d,l*-fenfluramine (20 mg/kg) or *d*-fenfluramine (10 mg/kg) produced slight decreases in the rate of body weight gain in Syrian hamsters over an eight day regimen, but there was no differences between hamsters that were running in wheels (mean activity, 14 km/night) or did not have access to wheels (sedentary). Fenfluramine may not have general effects on energy wasting.

Humans treated chronically with fenfluramine have increased serum levels of free fatty acids, ketones and glycerol, and decreased levels of triglycerides and total plasma lipids. The absorption of usual dietary levels of fat appears normal in fenfluramine-treated people, although there are substantial decreases in the rate of fat absorption in rats, which could be due to delayed absorption and/or decreased triglyceride synthesis in rat intestinal mucosa.

Fenfluramine produces rapid dose-related increases in serum corticosterone in rats. Exogenous administration of 5-HT has similar effects, and stimulates the release of corticotropin releasing hormone (CRH) from the hypothalamus, *d*-Fenfluramine also stimulates CRH by an apparently 5-HT-dependent mechanism.

Systemic administration of *d,l*-fenfluramine elevated serum corticosterone levels, but that this response was not affected by prior depletion of brain 5-HT with 5,7-DHT. The authors concluded that the effect of fenfluramine is indeed mediated by 5-HT, but via peripheral actions or direct post-synaptic effects. Likewise, the observation that chronic treatment with *d*-fenfluramine reduced the duration (but not the peak) rise in serum corticosterone of rats after a fructose load suggests peripheral actions. In contrast, brain 5-HT depletions with 5,7-DHT or by lesions of the raphe nucleus prevented the *d*-fenfluramine-induced increases in plasma corticosterone. The discrepant results raise the possibility that *d*- and *l*-fenfluramine release CRH by different mechanisms.

By far the most studied aspect of fenfluramine action involves the central nervous system. While it is well-established that fenfluramine affects 5-HT release and uptake in synaptosomal preparations, the contribution of these mechanisms *in vivo* to produce anorexia and/or tolerance is questionable. Clinical and experimental evidence in favour of an inhibitory role for brain 5-HT in feeding have been reviewed extensively. However, there is conflicting evidence, some of which indicates that brain 5-HT cannot be regarded as a unitary system in regard to fenfluramine action.

A decrease in binding of ³H-5-HT to some brain regions of rats treated for 28 days with *d*-fenfluramine (2.5 mg/kg twice daily) was observed. No such changes were evident after 14 days of treatment. Similar observations were reported with ³H-spiperone binding to type 2 serotonin receptors. However, others were unable to find any reliable change in ³H-5-HT binding after 28 days' chronic *d,l*-fenfluramine (5 mg/kg, twice daily) to rats. One way to reconcile these disparate results is if *l*-fenfluramine produces an opposite effect to *d*-fenfluramine.

Fenfluramine is known to inhibit the *in vitro* specific binding of 5-HT to brain membranes. The concentration which inhibits binding by 50% (IC₅₀) is highest for *d*-fenfluramine (about 7 microM) and *d*-norfenfluramine (4 microM), with the *l*-enantiomers effective at concentrations of about 2 microM.

While fenfluramine given acutely clearly causes 5-HT release above basal levels, for at least some time, an even more dramatic and long-lasting effect is its inhibition of 5-HT high affinity uptake into 5-HT-containing neurons. Because reuptake is probably the major route for inactivation of released 5-HT, inhibition of such uptake will clearly increase the synaptic concentrations of 5-HT and thus of post-synaptic receptor occupancy.

The enantiomers of fenfluramine and norfenfluramine have different IC₅₀ concentrations on uptake, *d*-Fenfluramine has the lowest (0.5 microM), some 3 x lower than *d*-norfenfluramine, and both are about 10x more potent than the corresponding *l*-enantiomers. There is thus clear evidence for a relationship between anorectic potency and inhibition of uptake, with *d*-fenfluramine potently inhibiting uptake at modestly anorectic doses.

Acute administration of fenfluramine or norfenfluramine releases 5HT from nerve terminals and so depletes brain 5-HT levels in Rats; the *d*- and *l*-enantiomers of fenfluramine appear to be equipotent in this regard. The decrease in 5-HT levels is prevented by serotonin uptake inhibitors such as chlorimipramine or fluoxetine and by the putative 5-HT agonist, quipazine. The effect of a single dose of fenfluramine can be long-lasting, depending upon the dose. Thus, it was found that 5-HT levels remained at a nadir for at least 6 h following 10 mg/kg *d,l*-fenfluramine in rats, but had returned to near normal by 24 h. However, after 15 mg/kg or

greater, brain 5-HT levels were at a minimum for at least 24 h and up to several weeks. Following chronic administration of fenfluramine at modest doses (<10 mg/kg daily), brain 5-HT levels recover within one week of ending the treatment.

The decreased 5-HT levels observed following fenfluramine injection (mostly in rats) are taken as evidence that release has occurred without compensatory replenishment of the storage vesicles (i.e., by synthesis and/or reuptake). The level in rat brain of the principal metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) is also reduced after fenfluramine. However, at least in the initial phase (1-2 h) after fenfluramine, the majority of studies report that 5-HIAA depletion lags behind that of 5-HT depletion. Pre-treatment with reserpine to deplete 5-HT storage granules greatly attenuated the 5-HT release to fenfluramine but not norfenfluramine, suggesting that the latter is releasing from a reserpine-insensitive pool. The release of 5-HT by fenfluramine is calcium-independent implying that neither autoreceptor processes nor impulse flow are needed.

The early phase of 5-HT release is followed by a prolonged period of decreased 5-HT activity, as indicated by decreased 5-HIAA. After high doses of fenfluramine this can be essentially permanent. The 5-HIAA/5-HT ratios often are slightly increased suggesting increased impulse flow once 5-HT is depleted.

For References - See Review

Rowland NE, Carlton J. *Neurobiology of an anorectic drug: fenfluramine. Prog Neurobiol.* 1986;27(1):13-62. Review. PubMed PMID: 3526413.

d-Fenfluramine decreased serotonin concentration in rat brain as early as 1 h; at 1 h 5-HIAA concentration was slightly increased, but at later times 5-HIAA was also decreased. At all time intervals studied, the 5-HIAA/serotonin ratio was increased by *d*-fenfluramine. Also, *d*-fenfluramine was unable to block the acute depletion of brain serotonin by *p*-chloroamphetamine. *d*-Fenfluramine also increased serum corticosterone and prolactin concentrations. The results supported the interpretation that *d*-fenfluramine initially enhances serotonergic function by carrier-dependent release of serotonin and not by uptake inhibition.

Fuller RW, Snoddy HD, Robertson DW. *Mechanisms of effects of d-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release. Pharmacol Biochem Behav.* 1988 Jul;30(3):715-21. PubMed PMID: 2463643.

Fenfluramine [(+/-)-fenfluramine, (+)-fenfluramine, (-)-fenfluramine] or its metabolites [(+/-)-norfenfluramine, (+)-norfenfluramine, (-)-norfenfluramine] were screened for activity at 11 cloned serotonin receptor subtypes by use of ligand-binding methods and functional assays. The fenfluramines and norfenfluramines had K_i values ranging from 673 to 1950 nmol/L and lacked agonist activity at the 5-HT_{1A} receptor (data not shown).

The fenfluramines and norfenfluramines had very low affinity for the 5-HT_{1D}, 5-HT_{1B}, 5-HT_{1D/1B} and 5-HT_{1E} receptors (data not shown). The ergot compounds had high affinity for the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (Table 1). The fenfluramines had micromolar affinity for the 5-HT_{2A} receptor.

The norfenfluramines were moderately potent at the 5-HT_{2C} receptor and were full agonists. The fenfluramines were also full agonists but were significantly less potent than the norfenfluramines. The norfenfluramines had high affinity (10 to 50 nmol/L) for the 5-HT_{2B} receptor, in confirmation of recent studies. Functional studies demonstrated that the norfenfluramines were full agonists at the 5-HT_{2B} site. The fenfluramines, in contrast, bound to the 5-HT_{2B} receptor with K_i values of 5 micro mol/L. The fenfluramines and norfenfluramines were inactive at the 5-HT₅ and 5-HT₆ receptors (data not shown) and have moderate affinity at the 5-HT₇ receptor (data not shown).

Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*. 2000 Dec 5;102(23):2836-41. PubMed PMID: 11104741.

TABLE 1. K_i Values of Test Drugs at 5-HT₂ Receptors

Drug	Rat 5-HT _{2A}	Human 5-HT _{2B}	Rat 5-HT _{2C}
(±)-Fenfluramine	5 216 ± 2.5	4134 ± 753	3183 ± 374
(+)-Fenfluramine	11 107 ± 1354	5099 ± 690	6245 ± 514
(-)-Fenfluramine	5463 ± 352	5713 ± 1344	3415 ± 542
(±)-Norfenfluramine	2316 ± 163	52.1 ± 12.3	557 ± 36
(+)-Norfenfluramine	1516 ± 88	11.2 ± 4.3	324 ± 7.1
(-)-Norfenfluramine	3841 ± 361	47.8 ± 18.0	814 ± 58
Ergotamine	9.0 ± 0.6	3.0 ± 0.2	12.0 ± 0.9
Methysergide	15.0 ± 2.4	9.1 ± 2.9	1.8 ± 0.1
Methylergonovine	12.6 ± 0.6	0.49 ± 0.09	12.4 ± 0.6
Fluoxetine	299 ± 31	5030 ± 1152	50.0 ± 5.9
Norfluoxetine	638 ± 63	5063 ± 1161	286 ± 35
Trazodone	19.8 ± 1.4	73.6 ± 21.17	402 ± 26
mCPP	391 ± 27	3.2 ± 0.6	59 ± 6.5
5-HT	614 ± 43	4.0 ± 1.1	12.2 ± 0.8
Phentermine	>10 000	>10 000	>10 000

Values are mean ± SEM (n=3). Units are nanomolar.

TABLE 2. Functional Activity of Test Drugs at 5-HT₂ Receptors

Drug	Human 5-HT _{2A}	Human 5-HT _{2B}	Human 5-HT _{2C}
(±)-Fenfluramine			
K_{50}	4 131 ± 14 400	ND	ND
V_{max}	15 ± 2.35		
(+)-Fenfluramine			
K_{50}	>10 000	379 ± 70	362 ± 64
V_{max}	ND	38 ± 8.2	80 ± 5.9
(-)-Fenfluramine			
K_{50}	5 279 ± 587	1248 ± 252	360 ± 91
V_{max}	43 ± 4.2	47 ± 2.9	84 ± 7.4
(±)-Norfenfluramine	ND	ND	ND
(+)-Norfenfluramine			
K_{50}	630 ± 141	18.4 ± 5.3	13 ± 2.4
V_{max}	88 ± 5.3	73 ± 3.5	100 ± 6.5
(-)-Norfenfluramine			
K_{50}	1 565 ± 190	357 ± 105	18 ± 5.3
V_{max}	93 ± 5.3	71 ± 8.8	80 ± 10
Ergotamine			
K_{50}	16 ± 2.3	9.8 ± 1.8	5 ± 1.76
V_{max}	75 ± 1.8	56 ± 1.8	75 ± 8.8
Methysergide			
K_{50}	3.5 ± 1.00	150 ± 25	2.9 ± 0.9
V_{max}	24 ± 1.76	18 ± 2.4	33 ± 2.0
Methylergonovine			
K_{50}	1.3 ± 0.2	0.8 ± 0.3	2.5 ± 0.7
V_{max}	70 ± 4.1	40 ± 1.8	103 ± 4.1
Fluoxetine	ND	ND	Antagonist $K_i = 616 ± 101$
Norfluoxetine	ND	ND	Antagonist $K_i = 43 ± 10$
Trazodone	Antagonist	Antagonist	Antagonist
mCPP			
K_{50}	65 ± 10	64 ± 15	0.64 ± 0.17
V_{max}	55 ± 6.5	43 ± 8.2	79 ± 8.8
5-HT			
K_{50}	66 ± 15	2.4 ± 0.9	0.6 ± 0.1
V_{max}	100	100	100
Phentermine			
K_{50}	ND	ND	1394 ± 264
V_{max}			66 ± 5.9

Values are mean ± SEM (n=3). K_{50} values are nmol/L ± SEM; V_{max} values are % of 5-HT ± SEM. ND indicates not done.

Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*. 2000 Dec 5;102(23):2836-41. PubMed PMID: 11104741.

[¹⁸F]MPPF is a selective and reversible antagonist to the 5-HT_{1A} receptor. [¹⁸F]MPPF binding was assessed using PET in conscious monkeys. Microdialyses results showed a 20- and 35-fold increase in extracellular 5-HT levels in the prefrontal cortex after injection of fenfluramine at a dose of 5 mg/kg and 10 mg/kg respectively. However, despite these large increases in 5-HT levels, no differences in binding potential were found between the control and fenfluramine scans. These results may imply that the majority of 5-HT_{1A} receptors is in the low affinity state in the living brain.

Udo de Haes JI, Harada N, Elsinga PH, Maguire RP, Tsukada H. Effect of fenfluramine-induced increases in serotonin release on [¹⁸F]MPPF binding: a continuous infusion PET study in conscious monkeys. *Synapse*. 2006 Jan;59(1):18-26. PubMed PMID: 16237679.

Two baboons and 1 rhesus monkey were given preblocking or displacing doses of fenfluramine (1-5 mg/kg) or preblocking doses of unlabeled P943 (0.2 mg/kg) and imaged

with [^{11}C]P943 PET. Receptor occupancy by the low dose of i.v. (\pm)-fenfluramine (1 mg/kg) in the baboons was 25 and 29% and by the high dose of (\pm)-fenfluramine (5 mg/kg) in the rhesus macaque was 42%. Receptor occupancy by P943 (0.2 mg/kg) was 68 and 86% in the baboons.

Cosgrove KP, Kloczynski T, Nabulsi N, Weinzimmer D, Lin SF, Staley JK, Bhagwagar Z, Carson RE. Assessing the sensitivity of [^{11}C]p943, a novel 5-HT_{1B} radioligand, to endogenous serotonin release. *Synapse*. 2011 Oct;65(10):1113-7. doi: 10.1002/syn.20942. Epub 2011 May 3. PubMed PMID: 21484884; PubMed Central PMCID: PMC3149753.

Six isoflurane-anesthetized baboons were scanned with [^{11}C] P943 PET at baseline, and following various pharmacological manipulations including S-(+)-fenfluramine administered intravenously. [^{11}C] P943 was observed to bind saturably and specifically to 5-HT_{1B} receptors and to be sensitive to all three challenges known to alter 5-HT levels in the proximity of receptors.

Ridler K, Plisson C, Rabiner EA, Gunn RN, Easwaramoorthy B, Abi-Dargham A, Laruelle M, Slifstein M. Characterization of *in vivo* pharmacological properties and sensitivity to endogenous serotonin of [^{11}C] P943: a positron emission tomography study in *Papio anubis*. *Synapse*. 2011 Nov;65(11):1119-27. doi: 10.1002/syn.20946. Epub 2011 May 19. PubMed PMID: 21538549.

PET study in cynomolgus monkeys with the new 5-HT_{1B} receptor radioligand [^{11}C]AZ10419369 is sensitive to fenfluramine-induced changes in endogenous serotonin levels *in vivo*. In a preliminary PET-study, the sensitivity of [^{11}C]AZ10419369 to altered endogenous 5-HT levels were examined. (\pm)-Fenfluramine-induced 5-HT release decreased radioligand binding in a dose-dependent fashion with a regional average of 27% after 1 mg/kg and 50% after 5 mg/kg.

In subsequent study, the effect of fenfluramine on [^{11}C]AZ10419369 binding potential was dose-dependent in the displacement paradigm and confirmed in the pretreatment paradigm. After intravenous pretreatment with (\pm)-fenfluramine (5.0 mg/kg), the mean binding potential of the occipital cortex decreased by 39%, from 1.38 ± 0.04 to 0.84 ± 0.09 . This study confirms that the new 5-HT_{1B} receptor radioligand [^{11}C]AZ10419369 is sensitive to fenfluramine-induced changes in endogenous serotonin levels *in vivo*.

Finnema SJ, Varrone A, Hwang TJ, Halldin C, Farde L. Confirmation of fenfluramine effect on 5-HT(1B) receptor binding of [(11)C]AZ10419369 using an equilibrium approach. *J Cereb Blood Flow Metab*. 2012 Apr;32(4):685-95. doi: 10.1038/jcbfm.2011.172. Epub 2011 Dec 14. PubMed PMID: 22167236; PubMed Central PMCID: PMC3318146.

Finnema SJ, Varrone A, Hwang TJ, Gulyás B, Pierson ME, Halldin C, Farde L. Fenfluramine –induced serotonin release decreases [^{11}C]AZ10419369 binding to 5-HT_{1B}-receptors in the primate brain. *Synapse*. 2010 Jul;64(7):573-7. doi: 10.1002/syn.20780. PubMed PMID: 20222157.

Interactions with Neurotransmitters

Dopamine

In common with amphetamine, d,l-fenfluramine was found to produce increases in striatal homovanillic acid (HVA) in rats and that there was no tolerance to this effect with several daily fenfluramine pre-treatments. DOPAC levels are also increased after fenfluramine. These changes are indicative of dopamine release by dexfenfluramine.

The apparent dopamine antagonist action of fenfluramine has been further supported by the fact that stereotyped biting and licking, but not sniffing induced by either the dopamine agonist apomorphine or the dopamine releaser amphetamine, is antagonized by fenfluramine, with the *l*-enantiomer more potent than the *d*-form.

d,l-Fenfluramine attenuates electrical self-stimulation of the lateral hypothalamus with no tolerance to this effect. Since self-stimulation behaviour is blocked by dopamine receptor antagonists, a role for DA receptor blockade in at least some of the actions of fenfluramine cannot be ruled out.

High doses of fenfluramine given acutely have been shown to cause small (< 20%) and transient depletions in brain DA content in rats, mice and hamsters.

Fenfluramine at concentrations of > 10 microM *in vitro* also causes dopamine release.

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Rowland NE, Carlton J. *Neurobiology of an anorectic drug: fenfluramine. Prog Neurobiol.* 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Norepinephrine

Norepinephrine is also released by fenfluramine. Thus, NE levels may show a transient decrease while turnover or *in vitro* release are increased. There is also evidence that fenfluramine inhibits NE uptake at < 10 microM.

There is evidence that acute fenfluramine weakly stimulates the peripheral sympathetic nervous system, but chronic fenfluramine may decrease plasma NE and other indices of sympathetic activation.

See Review

Rowland NE, Carlton J. *Neurobiology of an anorectic drug: fenfluramine. Prog Neurobiol.* 1986;27(1):13-62. Review. PubMed PMID: 3526413.

(+)-Fenfluramine, (-)-fenfluramine, (+)-norfenfluramine, and (-)-norfenfluramine released [³H]5-HT from synaptosomes with EC₅₀ values of 52, 147, 59, and 287 nM, respectively.

(+)-Fenfluramine and (+)-norfenfluramine released [³H] norepinephrine (NE) with EC₅₀ values of 302 and 73 nM.

Results from *in vivo* microdialysis experiments showed that intravenous injection of (+)-norfenfluramine elevates extracellular levels of 5-HT, NE, and dopamine (DA) in rat frontal cortex. The effects of (+)-norfenfluramine on NE and DA were antagonized by pretreatment with the NE uptake blocker nisoxetine. In summary, administration of fenfluramines can increase synaptic levels of 5-HT, NE, and DA in the cortex, and (+)-norfenfluramine likely contributes to these effects.

Rothman RB, Clark RD, Partilla JS, Baumann MH. (+)-Fenfluramine and its major metabolite, (+)-norfenfluramine, are potent substrates for norepinephrine transporters. *J Pharmacol Exp Ther.* 2003 Jun;305(3):1191-9. Epub 2003 Mar 20. PubMed PMID: 12649307.

Other Neurotransmitters

d-Fenfluramine elevates acetylcholine levels in striatum while *l*- and *d,l*-fenfluramine have little effect.

Glutamic acid and GABA have also been implicated insofar as there is a transient decrease in their uptake into synaptosomes after a high dose of fenfluramine.

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Rowland NE, Carlton J. *Neurobiology of an anorectic drug: fenfluramine. Prog Neurobiol.* 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Through a combination of functional neuroanatomy, feeding, and electrophysiology studies in rodents, it was reported that *d*-fenfluramine-induced anorexia requires activation of central nervous system melanocortin pathways. These results provided a mechanistic explanation of *d*-fenfluramine's anorexic actions and indicated that drugs targeting these downstream melanocortin pathways may prove to be effective and more selective anti-obesity treatments.

Specifically, it was observed that anorectic 5-HT drugs activate pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus and 5-HT_{2C}R is expressed on POMC neurons, which contributes to this effect. Moreover, 5-HT drug-induced hypophagia is attenuated by pharmacological or genetic blockade of downstream melanocortin 3 and 4 receptors.

Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD, Elmquist JK. Activation of central melanocortin pathways by fenfluramine. Science. 2002 Jul 26;297(5581):609-11. PubMed PMID: 12142539.

Heisler LK, Cowley MA, Kishi T, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro J, Zigman JM, Cone RD, Elmquist JK. Central serotonin and melanocortin pathways regulating energy homeostasis. Ann N Y Acad Sci. 2003 Jun;994:169-74. Review. PubMed PMID: 12851313.

Sleep and Arousal

Fenfluramine generally has a mild sedative effect. Thus, increases have been found by EEG analysis in total and slow wave sleep time cats (Foxwell et al., 1969; Funderburk et al., 1971; Johnson et al., 1971; Zolovick et al., 1973). However, in rats studied during the daytime, Fornal and Radulovacki (1983a,b) found increased latency to onset of sleep, and decreased total time in both slow wave and REM sleep. Rabbits showed little effect of fenfluramine on EEG (Funderburk et al., 1971). Non-human primates had unaltered activity and sleep latency (Tang and Kirch, 1971). There is a report of no change in pedometer activity of man after fenfluramine (Yudkin and Miller, 1971). Daytime drowsiness is, however, an often troublesome side-effect (e.g. Oswald et al., 1971; Pinder et al., 1975; Stunkard et al., 1973). However, since the nighttime effect of fenfluramine in man is reduced REM and increased transitions from deep to light slow wave sleep ("intrasleep restlessness") (Lewis et al., 1971), the daytime drowsiness could result, in part, from poor sleep by night. One interesting alternative is that the drug may have different mechanisms at different parts of the nycthemeral cycle.

See Review

Rowland NE, Carlton J. Neurobiology of an anorectic drug: fenfluramine. Prog Neurobiol. 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Adverse Effect

A systematic review of data from animal studies indicated that fenfluramines cause dose-related, long-lasting reductions in serotonin axonal markers in all the animal species tested and with all the routes of drug administration used. Doses of fenfluramines that produce signs of brain serotonin neurotoxicity in animals are on the same order as those used to treat humans for weight loss when one takes into account known relations between body mass and drug clearance.

McCann UD, Seiden LS, Rubin LJ, Ricaurte GA. Brain serotonin neurotoxicity and primary pulmonary hypertension from fenfluramine and dexfenfluramine. A systematic review of the evidence. JAMA. 1997 Aug 27;278(8):666-72. Review. PubMed PMID: 9272900.

Fenfluramine and dexfenfluramine can be associated with severe and persistent neuropsychiatric difficulties. As reported in case series with 31 patients, a variety of neuropsychiatric syndromes have been seen in individuals treated with the fenfluramines, including depression, mania, cognitive dysfunction, panic disorder, obsessive compulsive behaviour, insomnia, and poor impulse control.

In some patients, these disturbances are time-limited, and persist only while the individual is taking medication. In others, neuropsychiatric dysfunction persists well beyond the period of drug use, suggesting that humans, like animals may develop long-lasting serotonergic dysfunction after exposure to fenfluramines.

McCann UD, Eligulashvili V, Ricaurte GA. Adverse neuropsychiatric events associated with dexfenfluramine and fenfluramine. *Prog Neuropsychopharmacol Biol Psychiatry*. 1998 Oct;22(7):1087-102. PubMed PMID: 9829290.

Suicide and Depression

In pharmacological challenge tests, pharmacological substances are administered to the patient and neuroendocrine changes along with brain perfusion or metabolism changes are investigated. As an example, serotonin agonists, such as fenfluramine, are administered to the patients and an increase in the presynaptic release of serotonin is provoked. This process cannot be measured but its direct consequences can be assessed. Secondary and proportional to this post-synaptic receptor stimulation, the anterior pituitary gland releases prolactin in the circulation. If the serotonergic system is impaired a blunted increase in prolactin is found (Malone et al., 1996 and Correa et al., 2000). Besides an increase in prolactin, fenfluramine also increases frontal cortex metabolism (Soloff et al., 2003b).

See Review

Rowland NE, Carlton J. Neurobiology of an anorectic drug: fenfluramine. *Prog Neurobiol*. 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Suicide attempters exhibit a blunted release of prolactin in response to administration of fenfluramine, a measure of 5-HT activity.

Dulchin M. C, Oquendo M. A, Malone K. M, Ellis S. P, Li S, Mann J. J. Prolactin response to DL-fenfluramine challenge before and after treatment with paroxetine. *Neuropsychopharmacology*. 2001;25:395–401.

Duval F, Mokrani M. C, Correa H, Bailey P, Valdebenito M, Monreal J, Crocq M. A, Macher J. P. Lack of effect of HPA axis hyperactivity on hormonal responses to D-fenfluramine in major depressed patients: Implications for pathogenesis of suicidal behaviour. *Psychoneuroendocrinology*. 2001;26:521–537.

Malone K. M, Corbitt E. M, Li S, Mann J. J. Prolactin response to fenfluramine and suicide attempt lethality in major depression. *British Journal of Psychiatry*. 1996;168:324–329.

Mann J. J, McBride P. A, Malone K. M, DeMeo M. D, Keilp J. G. Blunted serotonergic responsivity in depressed patients. *Neuropsychopharmacology*. 1995;13:53–64.

Pandey G. N. Altered serotonin function in suicide. Evidence from platelet and neuroendocrine studies. *Annals of the New York Academy of Sciences*. 1997;836:182–200.

Weiss D, Coccaro E. F. Neuroendocrine challenge studies of suicidal behavior. *Psychiatric Clinics of North America*. 1997;20:563–579.

Prolactin responses to a single-dose challenge with fenfluramine (60 mg orally) were examined in 45 male patients with clearly defined major affective (n = 25) and/or personality disorder (n = 20) and in 18 normal male control patients. Prolactin responses to fenfluramine among all patients were reduced compared with responses of controls. Reduced prolactin responses to fenfluramine were correlated with history of suicide attempt in all patients but with clinician and self-reported ratings of impulsive aggression in patients with personality disorder only; there was no correlation with depression.

Coccaro EF, Siever LJ, Klar HM, Maurer G, Cochrane K, Cooper TB, Mohs RC, Davis KL. Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. *Arch Gen Psychiatry*. 1989 Jul;46(7):587-99. Erratum in: *Arch Gen Psychiatry* 1990 Feb;47(2):124. PubMed PMID: 2735812.

Indirect research results include a reduced 5-HIAA in cerebrospinal fluid in violent suicide attempters and a blunted increase in prolactin after a fenfluramine challenge.

Audenaert K, Peremans K, Goethals I, van Heeringen C. Functional imaging, serotonin and the suicidal brain. *Acta Neurol Belg*. 2006 Sep;106(3):125-31. Review. PubMed PMID: 17091615.

Healthy volunteers and depressed subjects were administered a fenfluramine and placebo challenge test at baseline and then followed for 2 years. Seven subjects made suicide attempts within the follow-up period. Both past and future attempters had lower total prolactin output (area under the curve) in response to fenfluramine relative to non-patients. Future attempters had lower cortisol response relative to all other groups. Lower cortisol response correlated with greater suicidal ideation 3 months and 1 year post-study. All subject groups reported a decrease in Profile of Mood States Fatigue subscale score and increase in finger tapping rate after receiving fenfluramine.

Keilp JG, Oquendo MA, Stanley BH, Burke AK, Cooper TB, Malone KM, Mann JJ. Future suicide attempt and responses to serotonergic challenge. *Neuropsychopharmacology*. 2010 Apr;35(5):1063-72. doi: 10.1038/npp.2008.28. Epub 2008 Mar 19. PubMed PMID: 18354392; PubMed Central PMCID: PMC3055397.

In fenfluramine challenge studies, depressed patients with comorbid PTSD have lower plasma cortisol compared to depressed patients without comorbid PTSD. Cortisol levels increase with age and the number of previous major depressive episodes is a predictor of the cortisol response to fenfluramine administration in depressed patients without PTSD, but not in depressed patients with comorbid PTSD.

Sher L. The concept of post-traumatic mood disorder. *Med Hypotheses*. 2005;65(2):205-10. PubMed PMID: 15922089.

Male, non-hospitalized combat-exposed veterans diagnosed with PTSD (DSM-III-R) and a similarly aged combat-exposed control group were assessed for both PTSD and depressive symptoms and prolactin responses to a 30-mg d-fenfluramine challenge test. There were no significant differences between the three groups (control, current PTSD, past PTSD) for baseline prolactin, peak prolactin, and time to reach peak, delta prolactin or area under the curve of the prolactin vs. time curve. Depressive symptoms and history of alcohol or tobacco abuse or dependence did not have a confounding effect on the prolactin responses to d-fenfluramine. This study suggests that a blunted prolactin response to d-fenfluramine may be a consequence of combat exposure rather than PTSD.

Schweitzer I, Morris P, Hopwood M, Maguire K, Norman T. Prolactin response to d-fenfluramine in combat-related post-traumatic stress disorder. *Int J Neuropsychopharmacol*. 2004 Sep;7(3):291-8. Epub 2004 Apr 26. PubMed PMID: 15122972.

Metabolic PET Studies

Concerning the involvement of the serotonergic system in suicidal behaviour, evidence comes from different imaging modalities. Brain metabolism studies, with fenfluramine as a challenging drug, pointed at reduced serotonergic function.

Using ¹⁸F-FDG PET and a challenge with oral fenfluramine were studied in patients with major depression and in healthy volunteers. The depressed subjects, two of whom were

suicide attempters, showed a blunted metabolic response to fenfluramine compared to the normal controls.

After *dl*-fenfluramine, healthy subjects had several areas of statistically significant increases in regional brain glucose metabolism, mostly in the left prefrontal and temporoparietal cortex, and areas of decreased metabolism, such as in the right prefrontal cortex. In contrast, the depressed patients had no areas of increase or decrease in metabolism, differing significantly from healthy subjects. Results with patients resembled those with healthy subjects who were scanned twice without active drug on either occasion.

Mann JJ, Malone KM, Diehl DJ, Perel J, Cooper TB, Mintun MA. Demonstration in vivo of reduced serotonin responsivity in the brain of untreated depressed patients. *Am J Psychiatry*. 1996 Feb;153(2):174-82. Erratum in: *Am J Psychiatry* 1996 Apr;153(4):588. PubMed PMID: 8561196.

Mann JJ, Malone KM, Diehl DJ, Perel J, Nichols TE, Mintun MA. Positron emission tomographic imaging of serotonin activation effects on prefrontal cortex in healthy volunteers. *J Cereb Blood Flow Metab*. 1996 May;16(3):418-26. PubMed PMID: 8621746.

Using ^{15}O -H₂O PET to assess changes in cerebral blood flow after intravenous D-fenfluramine in depressed female patients, when compared to female healthy volunteers, could not demonstrate any differences in response between the two groups. Interestingly, the authors suggested that the presence of suicidal behaviour among patients in the previous study by Mann in 1996 may have accounted for the decreased responsivity to fenfluramine. However, the findings can also be explained by the inclusion of only females in the second study's samples, and the fact that cerebral blood flow and not metabolism was measured.

Meyer JH, Kennedy S, Brown GM. No effect of depression on [(15)O]H₂O PET response to intravenous d-fenfluramine. *Am J Psychiatry*. 1998 Sep;155(9):1241-6. PubMed PMID: 9734549.

The brains of high lethality versus low lethality suicide attempters, most of whom having a depressive episode, with ^{18}F -FDG PET imaging were studied. This study showed relative hypometabolism in the high-lethality attempters compared to low-lethality attempters in the ventral, medial and lateral prefrontal cortex. This difference became more marked after fenfluramine challenge. A lower mean regional cerebral metabolic rate of glucose uptake (rCMRglu) correlated with higher lethality suicidal behaviour.

The authors also demonstrated that higher verbal fluency correlated positively with rCMRglu in the same regions of the prefrontal cortex and that lethality of the suicide attempt inversely correlated with prolactin after challenge. They found a lower CMRglu in high versus low-lethality suicide attempters. This hypometabolism in frontal cortex structures was related to the degree of suicide intent and impulsivity and not to depression (Oquendo et al., 2003).

Oquendo MA, Placidi GP, Malone KM, Campbell C, Keilp J, Brodsky B, Kegeles LS, Cooper TB, Parsey RV, van Heertum RL, Mann JJ. Positron emission tomography of regional brain metabolic responses to a serotonergic challenge and lethality of suicide attempts in major depression. *Arch Gen Psychiatry*. 2003 Jan;60(1):14-22. PubMed PMID: 12511168.

In a FDG-PET study, non-depressed borderline personality disorder subjects, most of whom had attempted suicide, had a lower relative regional increase in uptake of fluoro-deoxy glucose (FDG), in response to fenfluramine administration than controls in medial and orbital regions of right prefrontal cortex left middle and superior temporal gyri, left parietal lobe, and left caudate body.

Soloff PH, Meltzer CC, Greer PJ, Constantine D, Kelly TM. A fenfluramine-activated FDG-PET study of borderline personality disorder. *Biol Psychiatry*. 2000 Mar 15;47(6):540-7. PubMed PMID: 10715360.

The finding of blunted prefrontal activity after fenfluramine challenge is not a hallmark solely related to suicidal behaviour. In a ^{18}F -FDG PET study to evaluate the changes in regional glucose metabolism after fenfluramine or placebo administration, the impulsive-aggressive

patient population also showed significant blunted metabolic responses after fenfluramine administration in the orbital frontal, adjacent ventral medial, and cingulate cortex, compared to normal controls.

Siever LJ, Buchsbaum MS, New AS, Spiegel-Cohen J, Wei T, Hazlett EA, Sevin E, Nunn M, Mitropoulou V. *d,l-fenfluramine response in impulsive personality disorder assessed with [18F]fluorodeoxyglucose positron emission tomography. Neuropsychopharmacology. 1999 May;20(5):413-23. PubMed PMID: 10192822.*

A common finding in functional neuroimaging in resting conditions is a decreased perfusion in the prefrontal cortex of suicidal patients. During cognitive activation, relative lower increases in perfusion in the prefrontal cortex under the task have been observed. After fenfluramine challenge, the prefrontal cortex metabolism seemed to be inversely correlated to the lethality of previous suicide attempt.

In the future, neuroimaging may help to better understand the underlying neurobiological processes that lead to suicidal thoughts and the act of suicide by identifying dysfunctional brain regions or circuits. A better knowledge of the suicidal process will make researchers/clinicians able to intervene earlier in the suicidal process and ensure a better prediction and, more importantly, prevention of suicide in the future. Moreover, cerebral imaging has promising prospects concerning the evaluation of the effect of medication and psychotherapy.

Desmyter S, van Heeringen C, Audenaert K. *Structural and functional neuroimaging studies of the suicidal brain. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Jun 1;35(4):796-808. doi: 10.1016/j.pnpbp.2010.12.026. Epub 2011 Jan 6. Review. PubMed PMID: 21216267.*

Anxiety / Panic Disorder

In the social interaction test of anxiety, *l*-fenfluramine (2.5 and 5 mg/kg) significantly reduced the time spent in active social interaction, and decreased motor activity in rats. Analyses of covariance indicated that these were two independent effects.

In the elevated plus-maze, *l*-fenfluramine (1.25-5 mg/kg) significantly decreased the percent number of entries made onto open arms, and (2.5 and 5 mg/kg) significantly decreased the percent of times spent on the open arms. The total number of arm entries was reduced by all doses (0.625-5 mg/kg). These results indicating anxiogenic effects of fenfluramine in rats.

l-Fenfluramine (1.25 and 2.5 mg/kg) significantly reduced the success of dominant rats competing with untreated middle rank rats for chocolate. In resident rats, *l*-fenfluramine (2.5 mg/kg) significantly increased the number of submissions, and the time spent submitting, to untreated rats intruding into their home-cage territory; it also significantly reduced the number of kicks directed at, and the time spent kicking, the intruder; and the incidence of, and time spent in, aggressively grooming the intruder. When the intruder rats were treated with *l*-fenfluramine the only significant change was a decrease in the number of wrestling bouts and the time spent wrestling. Since *l*-fenfluramine did not change other behaviours in this test (e.g. sniffing the opponent) the decrease in dominance behaviours was probably not secondary to nonspecific sedation.

File SE, Guardiola-Lemaitre BJ. *l*-fenfluramine in tests of dominance and anxiety in the rat. Neuropsychobiology. 1988;20(4):205-11. PubMed PMID: 3255891.

The elevated T-maze test is intended to produce two types of fear in the same rat, conditioned fear being represented by inhibitory avoidance of the two open arms of the maze, and unconditioned fear by one-way escape from one of the open arms toward the enclosed arm, which is less aversive than the open arms. In this model of anxiety and

memory, fenfluramine increased escape latencies in a dose-dependent way, thus having an anxiolytic effect on unconditioned fear, but was anxiogenic on conditioned fear, since it tended to prolong inhibitory avoidance latency.

Graeff FG, Viana MB, Mora PO (1995) Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T maze. *Pharmacol Biochem Behav* 53:171–177

Acute administration of fenfluramine induced anxiety in panic disorder patients. However, the authors of this study pointed out *d*-fenfluramine causes a slow wave of anxiety that does not resemble the sudden surge that is characteristic of a true panic attack. An explanation compatible with the dual 5-HT – fear hypothesis is that in these challenge tests fenfluramine enhanced anticipatory anxiety, rather than triggered a panic attack.

Targum SD (1990) Differential responses to anxiogenic challenge studies in patients with major depressive disorder and panic disorder. *Biol Psychiatry* 28:2–34

Targum SD, Marshall LE (1989) Fenfluramine provocation of anxiety in patients with panic disorder. *Psychiatry Res* 28:295–306

Secretion curves for prolactin, cortisol, TSH, and GH from a 37-year old woman with dysthymia and panic disorder with agoraphobia were determined one day prior to (day I), and during a panic attack (day II) associated with an oral dose of 60 mg *d,l*-fenfluramine, a drug known to increase anticipatory anxiety. The increased cortisol secretion observed is discussed in relation to the hormonal correlates of anxiety and the possible role of depression, *d,l*-fenfluramine, and serotonergic receptor sensitivity.

Vieira AH, Ramos RT, Gentil V. Hormonal response during a fenfluramine-associated panic attack. *Braz J Med Biol Res.* 1997 Jul;30(7):887-90. Erratum in: *Braz J Med Biol Res* 1997 Sep;30(9):1145. PubMed PMID: 9361714.

In 12 normal healthy volunteers, fenfluramine produced different and apparently aversive effects (e.g., it increased measures of anxiety and confusion). Phentermine reduced the apparently aversive effects of fenfluramine when the two drugs were given together.

Brauer LH, Johanson CE, Schuster CR, Rothman RB, de Wit H. Evaluation of phentermine and fenfluramine, alone and in combination, in normal, healthy volunteers. *Neuropsychopharmacology.* 1996 Apr;14(4):233-41. PubMed PMID: 8924191.

Chronic administration of *d, l*-fenfluramine (60–180 mg/day for 3 months) in an open clinical trial reduced the frequency of panic attacks from 16.9 to 0.9 per month in a group of 18 female patients with a history of panic disorder of 22–23 years of duration.

Solyom L (1994) Controlling panic attacks with fenfluramine. *Am J Psychiatry* 151:621–622

A case series reported successful treating with fenfluramine of six panic disorder patients resistant or intolerant to conventional drug therapy.

Hetem LA (1996) Addition of *d*-fenfluramine to benzodiazepines produced a marked improvement in refractory panic disorder – a case report. *J Clin Psychopharmacol* 16:77–78

d-Fenfluramine was administered to healthy volunteers under two models of experimental anxiety; the first was a simulated public speaking test in front of a video camera, being evaluated mainly by self-rating scales including Norris Visual Analogue Mood Scale and the second was a conditioned fear test - the changes in skin electrical conductance caused by a tone associated once with an aversive white noise were measured. The doses of 15 and 30 mg fenfluramine capsules decreased anxiety induced by simulated public speaking test in a dose-dependent way.

In the conditioned fear test, however, the amplitude and level of skin conductance responses to the conditioned aversive stimulus were not significantly changed by fenfluramine. The results support the view that 5-HT exerts a dual action on brain mechanisms regulating anxiety, facilitating conditioned while inhibiting unconditioned fear. The presumed reduction in unconditioned fear caused by fenfluramine may have implications for the treatment of panic disorder.

Hetem LA, de Souza CJ, Guimarães ES, Zuardi AW, Graeff FG. Effect of d-fenfluramine on human experimental anxiety. *Psychopharmacology (Berl)*. 1996 Oct;127(3):276-82. PubMed PMID: 8912406.

d-Fenfluramine enhances anticipatory anxiety, whereas markedly decreasing the intensity of panic attacks induced by inhalation of 5% CO₂ in 13 drug-free patients with DSM-III-R panic disorder.

Mortimore C, Anderson IM. d-Fenfluramine in panic disorder: a dual role for 5-hydroxytryptamine. *Psychopharmacology (Berl)*. 2000 Apr;149(3):251-8. PubMed PMID: 10823406.

In an intruder Challenge study, 25 adult female cynomolgus monkeys housed for 2 years in stable social groups when exposed to an unfamiliar female of the same species for 20 min, monkeys that approached to within 1 m of the intruder (median latency to approach=3 min) were found to have significantly smaller prolactin responses to fenfluramine compared to "inhibited" animals that failed to approach the intruder ($t=2.9$, $df=23$, $P<0.009$; $rpb=-0.51$).

Manuck SB, Kaplan JR, Rymeski BA, Fairbanks LA, Wilson ME. Approach to a social stranger is associated with low central nervous system serotonergic responsivity in female cynomolgus monkeys (*Macaca fascicularis*). *Am J Primatol*. 2003 Dec;61(4):187-94. PubMed PMID: 14669270.

Learning and memory

Southgate et al. (1971) reported a dose-related decrease in the retention (measured by step-through latency) of a passive avoidance task in mice. This suggests that fenfluramine affects the consolidation and/or encoding aspects of the task. It was not tested whether this learning might be state-dependent.

Fenfluramine supports very good taste-aversion learning, an observation which suggests that not all forms of learning and memory are impaired.

Fenfluramine does impair conditional avoidance responding (Cox and Maickel, 1972; McElroy et al., 1982; Srimal et al., 1970) and escape from brain stimulation (Kornblith and Hoebel, 1976).

See Review

Rowland NE, Carlton J. Neurobiology of an anorectic drug: fenfluramine. *Prog Neurobiol*. 1986;27(1):13-62. Review. PubMed PMID: 3526413.

Fenfluramine produced deficits in fear retention as indicated by a notable lack of the immobility resulting from inescapable shocks. Depletion of central 5-HT neurones after long-term PCA treatment (2 X 10 mg/kg) completely blocked the retention impairment resulting from acute PCA (2.5 mg/kg) and fenfluramine (5 mg/kg).

Archer T, Ogren SO, Ross SB. Serotonin involvement in aversive conditioning: reversal of the fear retention deficit by long-term p-chloroamphetamine but not p-chlorophenylalanine. *Neurosci Lett*. 1982 Dec 23;34(1):75-82. PubMed PMID: 6219302.

Fenfluramine treatments, at doses higher than 1 mg/kg, produced retrograde amnesia in a one-trial appetitive learning task in rats.

Lalonde R, Vikis-Freibergs V. Manipulations of 5-HT activity and memory in the rat. *Pharmacol Biochem Behav.* 1985 Mar;22(3):377-82. PubMed PMID: 3873076.

The effects of modulating the serotonergic system with fenfluramine on short-term spatial memory were investigated in rats using delayed matching to position and delayed non-matching to position procedures. At 5 mg/kg, fenfluramine significantly affected latency to respond, total responses on the levers and nose pokes in the foodtray as well as accuracy, indicating a non-specific disruption of behaviour rather than a selective effect on memory processes.

Jansen JH, Andrews JS. The effects of serotonergic drugs on short-term spatial memory in rats. *J Psychopharmacol.* 1994 Jan;8(3):157-63. doi: 10.1177/026988119400800303. PubMed PMID: 22298582.

d-Fenfluramine, administered to neonatal rats on postnatal days 11-20, exhibited dose-related impairments of sequential and spatial learning and reference memory in the absence of sensorimotor impairments in adulthood. Developmental *d*-fenfluramine-induced spatial and sequential learning deficits are similar to previous findings with developmental MDMA treatment.

Morford LL, Inman-Wood SL, Gudelsky GA, Williams MT, Vorhees CV. Impaired spatial and sequential learning in rats treated neonatally with D-fenfluramine. *Eur J Neurosci.* 2002 Aug;16(3):491-500. PubMed PMID: 12193193.

The effect on hormonal output following forced swim and the effect on sequential learning in the Cincinnati water maze and spatial learning in the Morris maze beginning 3 days after *d,l*-fenfluramine administration were investigated in rats. Animals that received *d,l*-fenfluramine (15 mg/kg; four times every 2 h on a single day) had increased corticosterone and aldosterone titers following forced swim relative to control animals, although no differences in ACTH or testosterone were noted. Animals exposed to fenfluramine had lasting deficits in the Cincinnati water maze but not in the Morris water maze, regardless of testing order. These deficits in the Cincinnati water maze appear to be mediated by the elevation in adrenal output since adrenalectomy abolished the effect of fenfluramine. Corticosterone levels were shown to be elevated during the behavioural testing period in animals exposed to fenfluramine.

Williams MT, Morford LL, McCreary AE, Wood SL, Vorhees CV. Administration of D,L-fenfluramine to rats produces learning deficits in the Cincinnati water maze but not the Morris water maze: relationship to adrenal cortical output. *Neurotoxicol Teratol.* 2002 Nov-Dec;24(6):783-96. PubMed PMID: 12460661.

High-dose exposure of *d*-fenfluramine (5 mg/kg) in monkeys acutely disrupted the Operant Test Battery tasks consisting five food-reinforced tasks designed to model aspects of learning, short-term memory and attention, time estimation, motivation, and colour and position discrimination. One month after high-dose, short-course *d*-fenfluramine exposure, the sensitivities of the Operant Test Battery tasks to acute disruption by *d*-fenfluramine were essentially unchanged.

Frederick DL, Ali SF, Gillam MP, Gossett J, Slikker W, Paule MG. Acute effects of dexfenfluramine (*d*-FEN) and methylenedioxymethamphetamine (MDMA) before and after short-course, high-dose treatment. *Ann N Y Acad Sci.* 1998 May 30;844:183-90. PubMed PMID: 9668676.

Two doses (15 and 30 mg) *d*-fenfluramine were tested in six male and six female healthy volunteers according to a double blind, cross-over design. Plasma concentrations of prolactin and cortisol decreased after all treatments at 1 h and then increased post-drug to a peak at 4 h. Body temperature showed a similar pattern. *D*-fenfluramine was well tolerated with few side effects. It caused only minor sedation and little psychomotor impairment but there was some decrement in episodic memory. The 30 mg dose produced a mild anxiolytic and anti-aggressive action.

Bond AJ, Feizollah S, Lader MH. The effects of D-fenfluramine on mood and performance, and on neuroendocrine indicators of 5-HT function. *J Psychopharmacol.* 1995 Jan;9(1):1-8. doi: 10.1177/026988119500900101. PubMed PMID:22298686.

Fenfluramine resulted in impaired delayed spatial memory in normal humans completed a visuospatial working memory task. These effects were not due to nonspecific arousal, attentional, sensorimotor or perceptual changes.

Luciana M, Collins PF, Depue RA. Opposing roles for dopamine and serotonin in the modulation of human spatial working memory functions. *Cereb Cortex.* 1998 Apr-May;8(3):218-26. PubMed PMID: 9617916.

Twenty-eight children with mental retardation and ADHD took part in a double-blind, placebo-controlled, crossover study of fenfluramine and methylphenidate. Fenfluramine dosage was gradually increased to a standardized dose of 1.5 mg/kg per day, whereas methylphenidate was given in doses of 0.4 mg/kg per day. The children were assessed on laboratory tests of selective and sustained attention, visual matching, and colour matching, during which seat activity was monitored automatically. Results showed fenfluramine to be superior to placebo on the memory task, whereas methylphenidate reduced commission errors on a continuous performance test. Methylphenidate caused shorter response times, and fenfluramine caused increases, on two of the tests. Examiner behaviour ratings indicated significant improvements with both drugs on the domains of attention, activity level, and mood.

Aman MG, Kern RA, McGhee DE, Arnold LE. Fenfluramine and methylphenidate in children with mental retardation and attention deficit hyperactivity disorder: laboratory effects. *J Autism Dev Disord.* 1993 Sep;23(3):491-506. PubMed PMID: 8226583.

A double-blind cross-over study on the effect of fenfluramine in seven autistic boys over a period of 8 months demonstrated a significant decrease in blood serotonin levels during the fenfluramine phase in all subjects. Slight improvements were found in short-term auditory memory and some measures of receptive language skills, particularly in children functioning at a high level. There was no significant change in global psychometric measurements of general intelligence during therapy. No adverse clinical effect was observed. Fenfluramine may have some selective favourable effects on increasing attention in high-functioning autistic children.

Ho HH, Lockitch G, Eaves L, Jacobson B. Blood serotonin concentrations and fenfluramine therapy in autistic children. *J Pediatr.* 1986 Mar;108(3):465-9. PubMed PMID: 2936877.

In eight chronic schizophrenic subjects, neuropsychological and communicative functioning was worse after fenfluramine treatment, even though blood serotonin levels were about half those at baseline conditions.

Soper HV, Elliott RO Jr, Rejzer AA, Marshall BD Jr. Effects of fenfluramine on neuropsychological and communicative functioning in treatment-refractory schizophrenic patients. *J Clin Psychopharmacol.* 1990 Jun;10(3):168-75. PubMed PMID: 2198295.

Drug Addiction

Opiate

Tolerance to morphine analgesia was induced in male Sprague-Dawley rats by implantation of a morphine base pellet (75 mg, s.c.) on the first and second day and determining the magnitude of tolerance 72 h after the first implant by s.c. injection of a test dose of morphine (5 mg/kg). Implantation of a cocaine hydrochloride pellet, after the development of tolerance, blocked both the development and expression of morphine analgesic tolerance. Blockade of

morphine tolerance by cocaine was reinforced and facilitated by pretreatment with fenfluramine, whereas, acute administration of fenfluramine did not significantly affect morphine analgesia.

Misra AL, Pontani RB, Vadlamani NL. Blockade of tolerance to morphine analgesia by cocaine. *Pain*. 1989 Jul;38(1):77-84. PubMed PMID: 2780065.

Morphine increased tail-withdrawal latency in a dose-related manner in monkeys. The antinociceptive effects of morphine occurred with smaller doses when monkeys received fenfluramine. Fenfluramine attenuated the discriminative stimulus effects of morphine and this attenuation was prevented by 5-HT_{2A} receptor antagonist, MDL100907.

Li JX, Koek W, Rice KC, France CP. Effects of direct- and indirect-acting serotonin receptor agonists on the antinociceptive and discriminative stimulus effects of morphine in rhesus monkeys. *Neuropsychopharmacology*. 2011 Apr;36(5):940-9. doi: 10.1038/npp.2010.232. Epub 2011 Jan 5. PubMed PMID: 21209613; PubMed Central PMCID: PMC3077263.

Dexfenfluramine (0.5-2.5 mg/kg IP) consistently reduced heroin self-administration by Wistar rats at doses producing only modest decreases in food responding.

Higgins GA, Wang Y, Corrigan WA, Sellers EM. Influence of 5-HT₃ receptor antagonists and the indirect 5-HT agonist, dexfenfluramine, on heroin self-administration in rats. *Psychopharmacology (Berl)*. 1994 May;114(4):611-9. PubMed PMID: 7855223.

Dexfenfluramine (1 mg/kg) reduced heroin self-administration (heroin dose 0.03 mg/kg/infusion; FR5 schedule; 1-h session/day). This effect was antagonised by the 5-HT_{1/2} receptor antagonist metergoline (1 mg/kg). In the drug discrimination model, dexfenfluramine (0.5-2.5 mg/kg) produced no significant generalisation to a morphine cue, and also failed to modify the generalization curve to heroin. Dexfenfluramine (1 mg/kg) produced a slight decrement in response rate in the drug discrimination model and this effect was potentiated by heroin.

Higgins GA, Wang Y, Sellers EM. Preliminary findings with the indirect 5-HT agonist dexfenfluramine on heroin discrimination and self-administration in rats. *Pharmacol Biochem Behav*. 1993 Aug;45(4):963-6. PubMed PMID: 8415837.

Three series of experiments sought to investigate further the mechanism by which dexfenfluramine reduces heroin self-administration by male Wistar rats. In experiment 1, the effect of combined intravenous heroin and intraperitoneal dexfenfluramine injections on operant responding for food was examined. In experiment 2, the maintenance of dexfenfluramine suppression of heroin self-administration following chronic (7 day) treatment was evaluated. Finally, in experiment 3, the ability of various 5-HT antagonists to block the dexfenfluramine suppression was examined.

The results from experiment 1 suggest that sensorimotor deficits/malaise potentially associated with heroin/dexfenfluramine combinations are unlikely to account for the reductions in heroin self-administration. Experiment 2 suggested that the suppressant effect of dexfenfluramine on heroin responding may diminish rapidly following chronic treatment. Finally, central 5-HT₁ and/or 5-HT₂, but not 5-HT₃, receptors may underlie the suppressant effects of dexfenfluramine on heroin self-administration.

Wang Y, Joharchi N, Fletcher PJ, Sellers EM, Higgins GA. Further studies to examine the nature of dexfenfluramine-induced suppression of heroin self-administration. *Psychopharmacology (Berl)*. 1995 Jul;120 (2):134-41. PubMed PMID: 7480544.

In normal volunteers, oral fenfluramine (60 mg)/saline infusion significantly increased the analgesic potency of morphine during the opioid infusion, while fenfluramine alone produced

borderline analgesic effects. Fenfluramine alone decreased alertness slightly, but did not significantly increase morphine side effects.

Coda BA, Hill HF, Schaffer RL, Luger TJ, Jacobson RC, Chapman CR. Enhancement of morphine analgesia by fenfluramine in subjects receiving tailored opioid infusions. *Pain*. 1993 Jan;52(1):85-91. PubMed PMID: 8446441.

Healthy subjects receiving fenfluramine (60 mg orally) had a significantly higher increase in prolactin plasma levels than the controls. An opiate receptor antagonist, Naloxone infusion (15 mg) caused a significant reduction in the prolactin response to fenfluramine. Higher doses of naloxone (30 mg) do not further inhibit the prolactin secretion induced by fenfluramine.

Foresta C, Scanelli G, Indino M, Federspil G, Scandellari C. Naloxone reduces the fenfluramine-induced prolactin release in man. *Clin Endocrinol (Oxf)*. 1985 Apr;22(4):539-43. PubMed PMID: 3987071.

In healthy controls, naloxone, an opiate receptor antagonist, significantly reduced the clear-cut prolactin increase induced by fenfluramine. Whereas, in obese patients, an increment in prolactin levels (after fenfluramine), similar to that in the controls, was not affected by naloxone.

Argenio GF, Bernini GP, Vivaldi MS, del Corso C, Santoni R, Franchi F. Naloxone does not modify fenfluramine-induced prolactin increase in obese patients. *Clin Endocrinol (Oxf)*. 1991 Dec;35(6):505-8. PubMed PMID: 1769132.

Prolactin and cortisol responses to acute stimulation with d-fenfluramine were significantly blunted in detoxified heroin-dependent subjects with comorbid depressive disorders but not in detoxified addicts with aggressive behaviour and antisocial personality or with heroin addiction uncomplicated by other Axis I and II psychiatric disorders. Results suggest that the function of the serotonergic system is impaired in heroin addicts with comorbid depression but not in heroin addicts who are not clinically depressed. Thus, the serotonergic system does not appear to be impaired by prolonged opioid exposure, *per se*.

Gerra G, Fertonani G, Tagliavini P, Zaimovic A, Delsignore R, Maestri D, Avanzini P, Caccavari R, Brambilla F. Serotonin function in detoxified heroin abusers: prolactin and cortisol responses to fenfluramine challenge. *Psychiatry Res*. 1995 Sep 29;58(2):153-60. PubMed PMID: 8570767.

Prolactin and cortisol responses to acute stimulation with d-fenfluramine were significantly blunted in all depressive mothers of heroin addicts but not in mothers without psychopathological features. The addicted sons of depressed mothers also showed reduced prolactin and cortisol responses to fenfluramine. Results suggest that genetic 5-HT impairment is not involved in the pathogenesis of heroin addiction or codependence *per se*, and is probably linked to the presence of familial depression in comorbidity with the addictive disorder.

Gerra G, Caccavari R, Zaimovic A, Palladino M, Chittolini B, Giucastro G, Maestri D, Monica C, Fertonani-Affini G, Delsignore R, Brambilla F. Serotonergic function in mothers of opioid addicts: correlation with comorbid depression. *Acta Psychiatr Scand*. 1997 Jul;96(1):36-42. PubMed PMID: 9259222.

Prolactin and cortisol responses to acute stimulation with d-fenfluramine were significantly blunted in all detoxified heroin-dependent subjects with antisocial personality disorder or without other Axis I and II pathologies, 6 - 8 weeks after detoxification, in comparison with psychophysiologically healthy subjects.

Gerra G, Zaimovic A, Moi G, Bussandri M, Delsignore R, Caccavari R, Brambilla F. Neuroendocrine correlates of antisocial personality disorder in abstinent heroin-dependent subjects. *Addict Biol*. 2003 Mar;8(1):23-32. PubMed PMID: 12745412.

Cocaine

It has been suggested that the increase in serotonin transmission induced by indirect agonists such as fenfluramine and fluoxetine attenuates cue-elicited reinstatement of cocaine-seeking in rats through a 5-HT_{2C} receptor-dependent mechanism. In open label clinical studies, phentermine and fenfluramine are reported to reduce craving for alcohol and cocaine and to prevent relapse.

Instrumental responding for intravenous cocaine in rats at 85% of free-feeding weight was significantly decreased 50% by D-fenfluramine plus phentermine (D-Fen/Phen, 5 mg/kg of each for 1 day). A similar effect was obtained in normal-weight rats self-administering a cocaine-heroin mixture. Animals that were well trained to self-administer drug did not self-administer intravenous D-fenfluramine plus phentermine. It is suggested that effectiveness in reducing cocaine reinforcement is due in part to a satiating effect in which dopamine and acetylcholine are released in the nucleus accumbens.

Glatz AC, Ehrlich M, Bae RS, Clarke MJ, Quinlan PA, Brown EC, Rada P, Hoebel BG. Inhibition of cocaine self-administration by fluoxetine or D-fenfluramine combined with phentermine. Pharmacol Biochem Behav. 2002 Jan-Feb;71(1-2):197-204. PubMed PMID: 11812523.

Using immunocytochemistry and *in situ* hybridization, changes in c-fos in rat brain nerve cells of the caudate putamen and hypothalamus following acute cocaine or fenfluramine exposure were studied. Both drugs (20 mg/kg; i.p.) evoked rapid but transient increases in c-fos in the caudate putamen. In addition, Fos-like protein was expressed preferentially in striatal neurons containing the protein phosphatase inhibitor, DARPP-32.

In contrast, fenfluramine, but not cocaine, elicited c-fos mRNA and Fos-like protein in the neuroendocrine paraventricular nucleus (PVN) of the rat hypothalamus despite the fact that both drugs are known to be equally capable to stimulate the hypothalamic-pituitary-adrenal (HPA) axis. To further identify the phenotypes of nerve cells expressing c-fos by fenfluramine in the PVN, it was demonstrated that the immediate-early gene was induced in a subpopulation of neurons constitutively expressing nitric oxide synthase (NOS).

Torres G, Rivier C. Induction of c-fos in rat brain by acute cocaine and fenfluramine exposure: a comparison study. Brain Res. 1994 May 30;647(1):1-9. PubMed PMID: 8069690.

The effects of *d*-fenfluramine, on reinstatement of extinguished cocaine-seeking behaviour elicited by either response-contingent presentations of cocaine-paired cues or cocaine priming were examined in rats. Fluoxetine dose-dependently attenuated cocaine-seeking behaviour during extinction. Both fluoxetine and *d*-fenfluramine dose-dependently attenuated cue-reinstated cocaine-seeking behaviour. In contrast, neither drug reliably altered cocaine-seeking behaviour reinstated by cocaine priming.

Burmeister JJ, Lungren EM, Neisewander JL. Effects of fluoxetine and d-fenfluramine on cocaine-seeking behavior in rats. Psychopharmacology (Berl). 2003 Jul;168(1-2):146-54. Epub 2003 Jan 16. PubMed PMID: 12529808.

Rats that had been trained to press a lever for cocaine (0.75 mg/kg/0.1 ml, i.v.) paired with light and tone cues underwent daily extinction sessions during which responding had no consequences. Then the effects of different 5-HT receptor subtypes antagonists were examined with and without *d*-fenfluramine (1.0 mg/kg, i.p.) pretreatment on cue reinstatement. It was found that the 5-HT_{2C} receptor antagonist SB 242,084 (0-1.0 mg/kg, i.p.) reversed the *d*-fenfluramine-induced attenuation of cocaine cue-induced cocaine-seeking behaviours, while the 5-HT_{1A} receptor antagonist WAY100635 (0-1.0 mg/kg, s.c.) and the 5-HT_{2A} receptor antagonist ketanserin (0-10.0 mg/kg, i.p.) did not.

Burmeister JJ, Lungren EM, Kirschner KF, Neisewander JL. Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*. 2004 Apr;29(4):660-8. PubMed PMID: 14627998.

Acute administration of an appropriate dose combination of phentermine (which selectively releases dopamine/norepinephrine >> serotonin) decreased cocaine self-administration more than food-maintained responding in rhesus monkeys. Fenfluramine also selectively decreased cocaine-maintained responding, but only at the highest dose. Combining a lower dose of fenfluramine with phentermine selectively decreased cocaine-maintained responding, but not more than with phentermine alone. These results suggest that phentermine, as well as its combination with fenfluramine, may be useful in the treatment of cocaine abuse.

Glowa JR, Rice KC, Matecka D, Rothman RB. Phentermine/fenfluramine decreases cocaine self-administration in rhesus monkeys. *Neuroreport*. 1997 Apr 14;8(6):1347-51. PubMed PMID: 9172133.

Cocaine use appears to have an effect on the serotonergic mechanisms mediating prolactin release in humans. The prolactin responses to fenfluramine increased significantly in all 25 hospitalized male cocaine addicts the longer they were cocaine free. This effect was more pronounced in a subgroup of patients with a paternal history of alcoholism or drug abuse.

Buydens-Branchey L, Branchey M, Hudson J, Rothman M, Fergusson P, McKernin C. Serotonergic function in cocaine addicts: prolactin responses to sequential D,L-fenfluramine challenges. *Biol Psychiatry*. 1999 May 15;45(10):1300-6. PubMed PMID: 10349036.

In nineteen cocaine-dependent male inpatients, fenfluramine (60 mg) significantly reduced cocaine craving and increased cortisol and prolactin when compared with placebo.

Buydens-Branchey L, Branchey M, Hudson J, Rothman M, Fergusson P, McKernin C. Effect of fenfluramine challenge on cocaine craving in addicted male users. *Am J Addict*. 1998 Spring;7(2):142-55. PubMed PMID: 9598218.

The combination of phentermine and fenfluramine reduced cocaine withdrawal symptoms in an open trial.

Kampman KM, Rukstalis M, Pettinati H, Muller E, Acosta T, Gariti P, Ehrman R, O'Brien CP. The combination of phentermine and fenfluramine reduced cocaine withdrawal symptoms in an open trial. *J Subst Abuse Treat*. 2000 Jul;19(1):77-9. PubMed PMID: 10867304.

Hormone responses evoked by fenfluramine were examined in eight human cocaine users who resided on a closed research ward. Fenfluramine (60 mg oral) was given after a 7-day cocaine-free period and 3 days after a 5-day period of daily double-blind administration of intranasal cocaine (96 mg) and active placebo (4 mg cocaine). Cocaine significantly elevated plasma cortisol levels to a similar degree on the first and fifth days of administration, but did not alter prolactin levels on either day. The first fenfluramine challenge significantly increased plasma prolactin and cortisol, whereas the second challenge increased only prolactin. The reduction in fenfluramine-induced cortisol secretion after cocaine exposure suggests that deficits in 5-HT transmission during early cocaine abstinence might contribute to the maintenance of drug dependence.

Ghitza UE, Rothman RB, Gorelick DA, Henningfield JE, Baumann MH. Serotonergic responsiveness in human cocaine users. *Drug Alcohol Depend*. 2007 Jan 12;86(2-3):207-13. Epub 2006 Aug 23. PubMed PMID: 16930852.

Repeated smoked cocaine users (12-50 mg for 3 days) had a blunted prolactin and cortisol response to d-fenfluramine that lasted for at least 2 weeks of cocaine abstinence, which suppressed prolactin by 50% of baseline. The long-lasting and selective disruptions in

serotonin pathways following chronic cocaine use may provide a neurochemical basis for changes in mood commonly reported during cocaine withdrawal.

Haney M, Ward AS, Gerra G, Foltin RW. Neuroendocrine effects of d-fenfluramine and bromocriptine following repeated smoked cocaine in humans. *Drug Alcohol Depend.* 2001 Sep 1;64(1):63-73. PubMed PMID: 11470342.

Alcohol

Amphetamine and fenfluramine, administered alone, have been shown to reduce food and fluid intake as well as alcohol consumption while acute coadministration of these agents has been shown to suppress audiogenic seizure in rats withdrawn from alcohol.

Chronic coadministration of amphetamine (2 mg/kg) and fenfluramine (8 mg/kg) reduced alcohol consumption during choice trials in both alcohol-dependent and alcohol-nondependent rats while not affecting water intake. The findings indicate that coadministration of amphetamine and fenfluramine, a treatment effective in reducing alcohol withdrawal seizures, also selectively attenuates alcohol consumption.

Yu YL, Fisher H, Sekowski A, Wagner GC. Amphetamine and fenfluramine suppress ethanol intake in ethanol-dependent rats. *Alcohol.* 1997 Jan-Feb;14(1):45-8. PubMed PMID: 9014023.

At doses of 1 and 2 mg/kg, fenfluramine selectively reduced consumption of the alcohol-containing diet as compared to the isocaloric diets. Lower doses of fenfluramine blocked the increases in alcohol consumption induced by phentermine. In animals fed the nonalcoholic diet, the drug combination (2 mg/kg fenfluramine plus 8 mg/kg phentermine) produced a 63-82% reduction in consumption, an effect not seen when either drug was administered alone.

Neurochemical analysis from these animals revealed that the alcohol-dependent animals displayed a significant reduction of DOPAC and 5-HIAA levels in the striatum, frontal cortex, and hypothalamus after a 9-h withdrawal period, further implicating the serotonergic and dopaminergic systems in mediation of withdrawal symptoms and alcohol craving. Finally, 8 mg/kg phentermine plus 8 mg/kg fenfluramine completely abolished alcohol withdrawal seizures, compared to a 78% rate in saline treated rats.

Halladay AK, Fisher H, Wagner GC. Effects of phentermine and fenfluramine on alcohol consumption and alcohol withdrawal seizures in rats. *Alcohol.* 2000 Jan;20(1):19-29. PubMed PMID: 10680713.

Fenfluramine (0.625, 1.25, 2.5, and 5.0mg/kg; twice per day), at all doses tested, decreased ethanol intake (as measured using a two-bottle free-choice method) for swim-test susceptible line of rats that show a propensity for high-ethanol intake. A diet enriched with 3% tryptophan also significantly decreased ethanol intake.

West CH, Boss-Williams KA, Weiss JM. Effects of fenfluramine, 8-OH-DPAT, and tryptophan-enriched diet on the high-ethanol intake by rats bred for susceptibility to stress. *Alcohol.* 2011 Dec;45(8):739-49. doi: 10.1016/j.alcohol.2011.07.007. Epub 2011 Sep 15. PubMed PMID: 21924578.

Successful phentermine and racemic fenfluramine treatment of 150 obese patients led to the use of this therapy in 12 alcoholic patients. Eleven of 12 consecutive patients, most within hours, reported a total loss or marked decrease in alcohol craving. Their reported consumption of alcohol ceased or decreased markedly. The hypothesis is proposed that this treatment is successful because of the dual and balanced increase in the bioavailability of the neurotransmitters, dopamine and serotonin, in the nucleus accumbens.

Hitzig P. Combined dopamine and serotonin agonists: a synergistic approach to alcoholism and other addictive behaviors. *Md Med J.* 1993 Feb;42(2):153-6. PubMed PMID: 8097012.

d-Fenfluramine caused a significantly attenuated peak delta-prolactin response in the alcoholics relative to the controls ($p = 0.05$). A repeated-measures ANOVA of delta-prolactin yielded a significant within-subjects effect of time ($p < 0.05$), a within-subjects effect of group that reached significance. There was also some evidence for a diminished serotonergic response in those alcoholics with a negative family history.

Farren CK, Ziedonis D, Clare AW, Hammeedi FA, Dinan TG. *D-fenfluramine-induced prolactin responses in postwithdrawal alcoholics and controls. Alcohol Clin Exp Res.* 1995 Dec;19(6):1578-82. PubMed PMID: 8749830.

With *d*-fenfluramine challenge test, there was no difference in baseline prolactin concentrations between tobacco-using and tobacco-nonusing alcohol-dependent individuals. On the other hand, the maximum prolactin response after *d*-fenfluramine was significantly lower in the tobacco-using group as compared to the tobacco-nonusing individuals.

Berggren U, Fahlke C, Eriksson M, Balldin J. *Tobacco use is associated with reduced central serotonergic neurotransmission in type 1 alcohol-dependent individuals. Alcohol Clin Exp Res.* 2003 Aug;27(8):1257-61. PubMed PMID: 12966319.

Controlling for gender and aggression, prolactin responses to fenfluramine administration in patients with major depression with co-occurring alcohol use disorders were significantly lower compared to the healthy control group.

Sher L, Stanley BH, Cooper TB, Malone KM, Mann JJ, Oquendo MA. *Serotonergic responses in depressed patients with or without a history of alcohol use disorders and healthy controls. Eur Neuropsychopharmacol.* 2008 Sep;18(9):692-9.doi: 10.1016/j.euroneuro.2008.05.005. Epub 2008 Jun 30. PubMed PMID: 18590952.

In a PET study, severe hypofrontality was found in an anterior medial prefrontal cortical area in patients with major depressive disorder and comorbid alcohol dependence compared to major depressive disorder only patients. This area encompassed the left medial frontal and left and right anterior cingulate gyri. This group difference disappeared after fenfluramine administration.

Sher L, Milak MS, Parsey RV, Carballo JJ, Cooper TB, Malone KM, Oquendo MA, Mann JJ. *Positron emission tomography study of regional brain metabolic responses to a serotonergic challenge in major depressive disorder with and without comorbid lifetime alcohol dependence. Eur Neuropsychopharmacol.* 2007 Sep;17(9):608-15. Epub 2007 May 2. PubMed PMID: 17478085.