

# Stress-Induced Hyperthermia, the Serotonin System and Anxiety

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**Abstract:** The serotonin (5-HT) system plays a key role in the pathophysiology of psychiatric disorders including mood and anxiety disorders. A role for serotonin in stress-related disorders is further supported by the fact that clinically effective treatments for these disorders alter serotonergic neurotransmission. The therapeutic potential of serotonergic pharmacological interventions has resulted in a variety of preclinical approaches to study the serotonin system. Of these, the stress-induced hyperthermia (SIH) paradigm has been extensively used to study the serotonin system at a preclinical level. The SIH response uses the transient rise in body temperature in response to a stressor which can be reduced using anxiolytic drugs including benzodiazepines, CRF receptor antagonists and serotonergic ligands.

The present review aims to discuss the acute and chronic effects of 5-HT ligands on the SIH response. Also, the SIH response in genetically modified mice that lack or overexpress specific serotonergic receptor subtypes or the serotonin transporter will be summarized. 5-HT<sub>1A</sub> receptor ligands reduce the SIH response, whereas acute administration of other serotonergic drugs (including 5-HT<sub>1B</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> modulators and SSRIs) generally does not influence the SIH response. Also, the SIH paradigm is generally insensitive to detect the anxiolytic effects of chronic serotonergic antidepressants in rodents, and serotonergic drugs that have been found to reduce the SIH response acutely do so irrespective of the healthy or pathological status of an individual.

**Keywords:** Stress, serotonin, 5-HT, SERT, autonomic nervous system, model.

## 1. INTRODUCTION

The serotonin (5-HT) system is important in the pathophysiology of psychiatric disorders including mood and anxiety disorders [1]. Specifically, the serotonergic system has been implicated in changes that are present in stress-related disorders including alterations in appetite, sleep, mood, and cognition. In support, depressed patients have decreased plasma levels of tryptophan [2, 3] and decreased cerebrospinal fluid levels of the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA) [4, 5]. A role for serotonin in stress-related disorders is further supported by the fact that clinically effective treatments for these disorders alter serotonergic neurotransmission. Selective serotonin reuptake inhibitors (SSRIs) increase serotonergic signaling by blocking the serotonin transporter (SERT) and are widely used in anxiety disorders and major depressive disorder [6]. Although evidence points at a dysfunctional serotonin

system in depression and anxiety disorders, the underlying causes of these disorders are complex and may also involve other neurotransmitter systems including the noradrenalin and dopamine systems [7, 8]. Moreover, serotonin can modulate the dopaminergic and noradrenalin systems, and *vice versa* [9-12]. Therefore, recent efforts have been made to develop serotonin-noradrenalin-dopamine reuptake inhibitors (which block the transporters for all three key biogenic amines, so-called "triple reuptake inhibitors") for the treatment of stress-related disorders [13].

The therapeutic potential of serotonergic pharmacological interventions has resulted in a variety of preclinical approaches to study the serotonin system. The stress-induced hyperthermia (SIH) paradigm has been extensively used to study the serotonin system at a preclinical level [14, 15]. The SIH response uses the transient rise in body temperature in response to a stressor which can be reduced using anxiolytic drugs including benzodiazepines and serotonergic ligands [16]. Of the different neurotransmitter systems, the serotonin system has received ample attention in the SIH paradigm. Therefore, the present review aims to discuss the acute and chronic effects of serotonergic ligands on the SIH response as well as to present an overview of the SIH response and

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drug responsiveness of genetically modified mice that lack or overexpress specific 5-HT receptor subtypes or the SERT.

## 2. THE SIH PARADIGM

The first use of SIH in anxiety research occurred after it was noted that removing mice one by one from a group-housed cage increased body temperature of the last mouse compared to the first (the group-housed version of the SIH paradigm, G-SIH) [14]. Later on, this paradigm was refined to a singly-housed version in which the rectal temperature was measured twice with an interval of 10 min [17]. Here, the first rectal temperature measurement ( $T_1$ ) is the basal unstressed core temperature but also functions as a stressor, whereas the second rectal temperature measurement ( $T_2$ ) is the stress-induced body temperature which is increased due to the stress experienced from the first temperature measurement. The difference in temperature ( $\Delta T = T_2 - T_1$ ) is defined as the SIH response. A typical SIH response differs from species to species, but may range from 0.5 to 2°C. More recently, telemetric setups have enabled the continuous registration of body temperature responses to stress which opens up possibilities for more advanced SIH studies [18, 19]. In anxiety research, it is difficult to find models with sufficient clinical predictive validity to support the translation of animal studies on anxiolytic drugs to clinical research. The highly reproducible and robust SIH response, combined with ease of testing, make the SIH paradigm very suitable for drug screening. The SIH response is larger and longer-lasting more with increased stressor intensity in which case both the amplitude and the duration of the SIH increase [20]. This phenomenon is limited by a physiological temperature ceiling value above which no further stress-induced temperature rise is possible. Using stressors with increasing intensity (disturbance stress by entering the room; handling stress; handling and injection stress; novel cage stress) the amplitude but in particular the duration of the SIH increases (up to 120 min), even though clear strain differences are present [20]. The effects of drugs on basal body temperature can hamper the interpretation of SIH data as severe hypothermia can interfere with thermoregulatory homeostatic processes. Together, it can be concluded that the SIH response should be contextually interpreted and depends on stressor type and intensity.

## 3. SEROTONIN RECEPTORS AND THE SEROTONIN TRANSPORTER

### 3.1. The Serotonin System

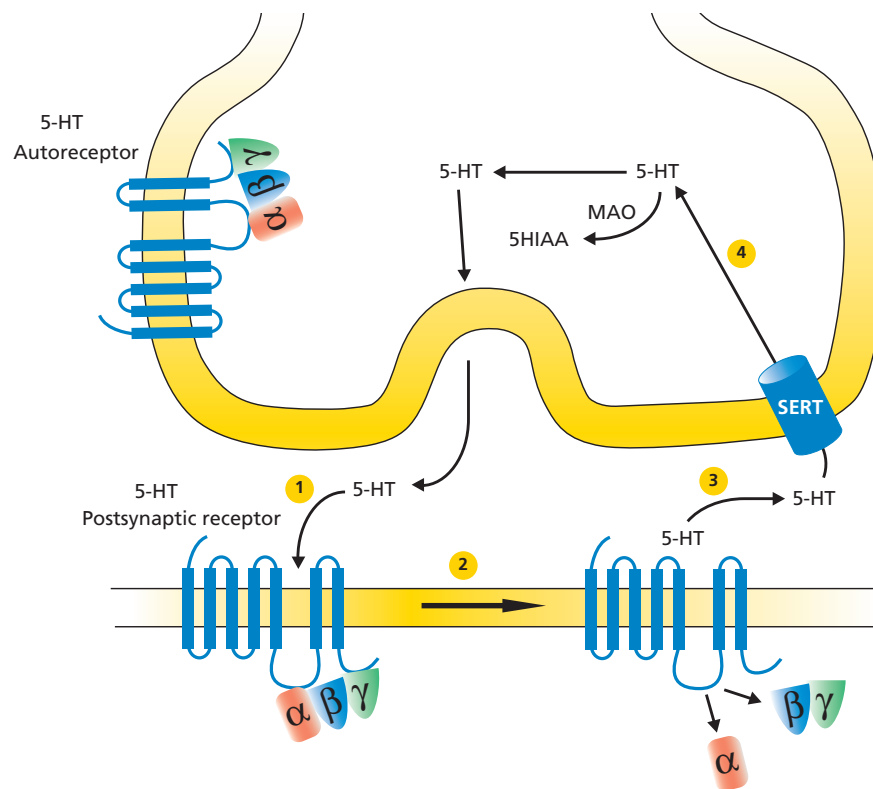
Serotonergic effects are mediated by multiple receptor subtypes with distinct distribution, localization, receptor structure, and second messenger systems that exert many functions in the central nervous system [21]. The 5-HT receptor family consists of at least 14 structurally and pharmacologically distinct receptors, of which 13 are G-protein-coupled receptors and one (the 5-HT<sub>3</sub> receptor) is a ligand-gated ion channel. Serotonergic neurons in the dorsal and median raphe nuclei of the brain stem are the main source of serotonin in the central nervous system. Serotonin, which is synthesized *via* tryptophan hydroxylase and

L-amino acid decarboxylase, binds to all these different pre- and postsynaptic 5-HT receptors (Fig. 1). Serotonergic receptors are distributed throughout the CNS, with a preferential presynaptic location in the raphe nuclei and a postsynaptic location in various limbic structures including the amygdala and the hippocampus [22-24]. In addition to a general pre- and postsynaptic distribution, 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors are also found as somatodendritic autoreceptors in the raphe nuclei which are pivotal in the control of serotonergic output to the frontal cortex as well as the hippocampus and is thought to be involved in the effects of SSRIs [25]. Moreover, the fact that specific 5-HT receptor subtypes may exist in different isoforms (after gene splicing or post-transcriptional processes) indicates the true complexity of the 5-HT system [26]. Another important modulator of the 5-HT system is the serotonin transporter (SERT), an integrated membrane protein which is both localized at the terminal portion of the axon as well as at the cell body of 5-HT neurons [27-29]. The SERT is pivotal in the reuptake of serotonin from the extracellular space into the presynaptic neuron, regulating the extracellular serotonin concentration and thus affecting the 5-HT system. After reuptake, 5-HT is stored in vesicles or is degraded *via* the monoamine oxidase enzyme to 5-hydroxy-indole acetaldehyde (5-HIAA) [30].

### 3.2. The Role of the 5-HT Receptor Family in Stress and Anxiety

Serotonergic receptors play a pivotal role in the modulation of behavioral, autonomic and endocrine stress responses. Of the serotonin receptor subtypes, the 5-HT<sub>1A</sub> receptor has been suggested to play a pivotal role in the pathophysiology of anxiety and depression [31, 32]. Specifically, depression is associated with presynaptic 5-HT<sub>1A</sub> receptor upregulation and postsynaptic 5-HT<sub>1A</sub> receptor downregulation [33]. Generally, 5-HT<sub>1A</sub> receptor agonists exert anxiolytic actions in rodents and humans [1], and genetically modified 5-HT<sub>1A</sub> receptor knockout mice display increased anxiety behavior [34-36]. Analysis of postmortem 5-HT<sub>1A</sub> receptor levels in humans after suicide have yielded inconclusive results ranging from an increase in 5-HT<sub>1A</sub> receptor binding [37-39], to no differences [40-43]. A recent postmortem study found decreased binding of the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 to the orbitofrontal cortex in major depressive disorder patients [44]. Imaging studies confirm that the WAY-100635 binding potential is significantly decreased in depressed patients [45], although the evidence is inconsistent [46]. The delay in the onset of SSRI efficacy may be ascribed to developing 5-HT<sub>1A</sub> receptor desensitization which may be pivotal to establish a clinical response to SSRIs. This idea is supported by the fact that co-administration of the beta-adrenergic/5-HT<sub>1A</sub> receptor antagonist pindolol may decrease SSRI latency of onset [47].

In addition to 5-HT<sub>1A</sub> receptors, animal studies have implicated 5-HT<sub>1B</sub> receptors in the development of stress-related disorders. However, the exact involvement of the 5-HT<sub>1B</sub> receptor subtype in stress-related disorders is unclear due to the lack of a receptor-specific ligand. 5-HT<sub>1B</sub> receptors are found as autoreceptors regulating the release of 5-HT and also act as heteroreceptors on non-serotonergic



**Fig. (1).** Schematic representation of serotonin (5-HT) in the terminal and synapse. G-protein coupled receptors are located presynaptically (5HT autoreceptor (5-HT<sub>1A/1B</sub>) or postsynaptically (5-HT<sub>1/2/4/5/6/7</sub> receptors). 1) 5-HT is released from presynaptic neuron and binds to heterotrimeric G-protein postsynaptic receptor. Heterotrimeric G protein complexes contain an alpha, beta and gamma subunit, which in the inactive state are bound to GDP. 2) 5-HT acts on postsynaptic receptor and a change in the conformation of the postsynaptic receptor is induced. GDP is fosforylated to GTP and binds to the  $\alpha$  subunit, which then becomes active. The  $\beta$  and  $\gamma$  subunits are freed. 3) Extracellular 5-HT is taken up by the SERT into the presynaptic neuron. 4) Back in the presynaptic neuron 5-HT is broken down by MOA to 5-HIAA or is being stored fore future release. MAO: Mono-amine oxidase. SERT: serotonin transporter; 5HIAA: 5-hydroxyindole acetic acid.

neurons [48, 49]. Studies on the involvement of 5-HT<sub>1B</sub> receptors in anxiety-related behavior are contradictory. 5-HT<sub>1B</sub> receptor agonists show anxiogenic-like effects [50, 51] and overexpression of 5-HT<sub>1B</sub> autoreceptors results in increased basal but reduced stress-induced anxiety-like behavior in rats [52, 53]. In contrast, higher 5-HT<sub>1B</sub> autoreceptor mRNA levels are correlated with decreased anxiety-like behavior [53-55]. Moreover, 5-HT<sub>1B</sub> receptor knockout mice do not display clear alterations in anxiety-like behavior, even though altered autonomic stress responses have been reported [56, 57]. Altogether, the role of 5-HT<sub>1B</sub> receptors in anxiety and depression is at best circumstantial and more research is necessary to draw conclusions on the involvement of these receptors in stress-related disorders. A far more convincing role for the 5-HT<sub>1B</sub> receptor is the involvement in impulse control and aggressivity that has been convincingly demonstrated [58-61].

The precise function of 5-HT<sub>2</sub> receptors in anxiety states is complex. There are studies that report 5-HT<sub>2A</sub> receptor upregulation on platelets in depression [62-64], whereas others have reported a downregulation [65]. Therefore, it may be hypothesized that the effects of a dysfunctional 5-HT<sub>2A</sub> system may differentially affect receptor levels depending on the brain structure. In support, expression of the 5-HT<sub>2A</sub> receptor in the cortex of 5-HT<sub>2A</sub> knockout mice (with an increased anxiety-like behavior) normalized anxiety

levels, suggesting that 5-HT<sub>2A</sub> receptors in the cortex are involved in anxiety-related processes [66]. So far, brain imaging studies have shown mixed results [33]. Activation of 5-HT<sub>2C</sub> receptors, including administration of agonists like 1-(m-Chlorophenyl)piperazine (mCPP) and 6-Chloro-2-(1-piperazinyl)pyrazine (MK-212) results in anxiogenic and panic-like responses in humans [67-69] as well as animals [70-72], even though this seems to be dose-dependent [73]. In line with this observation, 5-HT<sub>2C</sub> receptor antagonists have been reported to exert anxiolytic effects [74-77] and 5-HT<sub>2C</sub> knockout mice show decreased anxiety-like behavior [78]. Interestingly, anxiogenic behavior induced by acute administration of SSRIs (e.g. fluoxetine) may be blocked by 5-HT<sub>2C</sub> receptor antagonists, indicating that acute anxiogenic effects that acutely occur after SSRIs may be attributed to stimulation of the 5-HT<sub>2C</sub> receptor [79]. Overall, both 5-HT<sub>2A</sub> as well as 5-HT<sub>2C</sub> receptors are implicated in anxiety-like behavior and the contribution to an overall dysfunctional 5-HT system in various psychiatric disorders. As 5-HT<sub>2</sub> receptors are known shown to modulate other neurotransmitter systems including GABAergic [1], glutamatergic [80, 81] and dopaminergic neurons [82], 5-HT<sub>2</sub> receptor-mediated effects may be at least partially attributable to the downstream modulation of other neurotransmitter systems.

The 5-HT<sub>3</sub> receptor is a ligand-gated ion channel in contrast to the other G-protein-coupled 5-HT receptors [83].

Putative anxiolytic effects have been attributed to 5-HT<sub>3</sub> receptor antagonists which may be mediated through 5-HT<sub>3A</sub> receptors in limbic structures [84-86]. However, knockout studies have not yielded a clear role for the 5-HT<sub>3A</sub> receptor in the regulation of anxiety and depression [87]. The contributions of 5-HT<sub>3A</sub> receptors may alternatively affect the genetic vulnerability to develop a dysfunctional 5-HT system, and humans carrying an allelic variation (single nucleotide polymorphism) in the 5-HT<sub>3A</sub> receptor gene (C178T) exhibit lower scores for anxiety-related traits [88]. Overall, the exact role of the 5-HT<sub>3</sub> receptors in stress-related disorders has not been fully elucidated yet but does not seem to present a breakthrough in the treatment of anxiety disorders.

5-HT<sub>4</sub>, 5-HT<sub>5</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors have received less attention compared to the other serotonergic receptors. Nonetheless, there is some evidence that some of these receptors may be involved in anxiety processes. Antagonizing or knocking out 5-HT<sub>4</sub> receptors reduces anxiety like behavior [89, 90], although conflicting results exist [91]. No clear effects were found after knocking out 5-HT<sub>5A</sub> receptors in mice [92]. Pharmacological antagonism of 5-HT<sub>6</sub> receptors is known to produce anxiolytic-like effects [93-95], although no anxious phenotype was found in 5-HT<sub>6</sub> receptor knockout mice [96]. 5-HT<sub>7</sub> receptor antagonists exert anxiolytic effects [97], and 5-HT<sub>7</sub> receptor knockout mice do not display altered anxiety-like behavior [98, 99]. In the various studies that address the role 5-HT<sub>4,5,6,7</sub> receptors, there appears to be a discrepancy between pharmacological (anxiolytic effect) and genetic knockout studies (no anxiolytic effect), indicating that these receptors may not be constitutionally necessary to develop a normal anxiety-related phenotype.

Beside 5-HT receptors, the SERT may also impact anxiety-like behavior. The SERT modulates the magnitude and duration of action of serotonin on both pre- and postsynaptic 5-HT receptors. Knocking out the SERT gene or pharmacologically blocking the SERT increases anxiety-like behavior in mice [100-102] and rats [103]. Moreover,

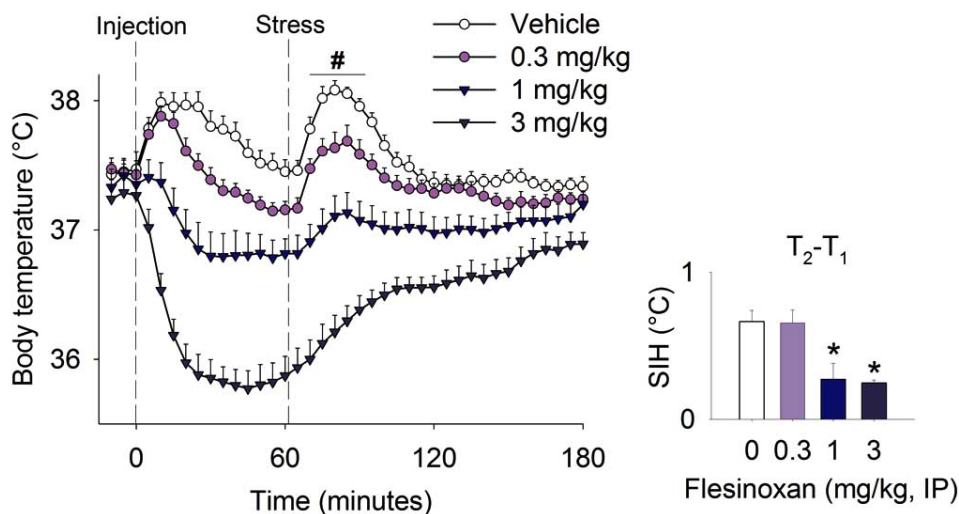
the link between the polymorphism in the promoter region of the human SERT gene (5-HTTLPR) and stress-related disorders has been established [104], although a recent meta-analysis yielded no evidence for an interaction between 5-HTTLPR genotype and adult stress-related behaviors [105]. Thus, if genetic vulnerability to depression is at least partially attributed to allelic variations in genes that influence the 5-HT system, such a predisposition to develop a dysfunctional 5-HT system seems to be not the sole explaining factor in the development of stress-related disorders.

Altogether, the majority of the 5-HT receptor subtypes may be differentially involved in normal and stress-related behaviors. Of these different 5-HT receptors, the 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor have been consistently shown to be closely involved in stress-related behavior. A complex interaction between the various 5-HT receptors, the SERT and other neurotransmitter systems is apparent, and it is beyond doubt that these interactions yield an extremely fine-tuned system. Therefore, it may not be surprising that disturbances in this system may lead to the development of psychiatric disorders.

#### 4. EFFECTS OF SEROTONERGIC COMPOUNDS ON THE SIH RESPONSE

##### 4.1. Effects of 5-HT<sub>1A</sub> Receptor Ligands on the SIH Response (Table 1)

In line with a prominent role for the 5-HT<sub>1A</sub> receptor in anxiety processes, the studies on serotonergic drugs in the SIH paradigm has focused on 5-HT<sub>1A</sub> receptor agonists. The 5-HT<sub>1A</sub> receptor agonist flesinoxan has received ample attention as an anxiolytic attenuating the SIH response [106-110]. In rodents, flesinoxan generally reduces the SIH response at lower doses, although hypothermia at higher doses makes the anxiolytic effects less readily interpretable. In Fig. (2), a typical example is shown of the SIH-reducing and hypothermic effects of flesinoxan in rats. Also, other 5-HT<sub>1A</sub> receptor agonists like 8-OH-DPAT [111] and flibanserin [112], have also been shown to attenuate the SIH response. Using the classical group-housed SIH test,



**Fig. (2).** Acute administration of the 5-HT<sub>1A</sub> receptor agonist flesinoxan (0-3 mg/kg, IP) reduced the SIH response as well lowered basal body temperature in Wistar rats (n=12). Error bars represent the S.E.M. \*: significant reduction of the SIH response (p<0.05); #: overall drug effect on basal body temperature (p<0.05). Unpublished data.

**Table 1. Effects of 5-HT<sub>1A</sub> Receptor Ligands on Basal Body Temperature (T<sub>1</sub>, Hypothermia) and the Stress-Induced Hyperthermia (SIH) Response. G-SIH: Group-House SIH Paradigm, Including the Injection-Stressor Interval (Minutes). PO: Oral, IP: Intraperitoneal, SC: Subcutaneous**

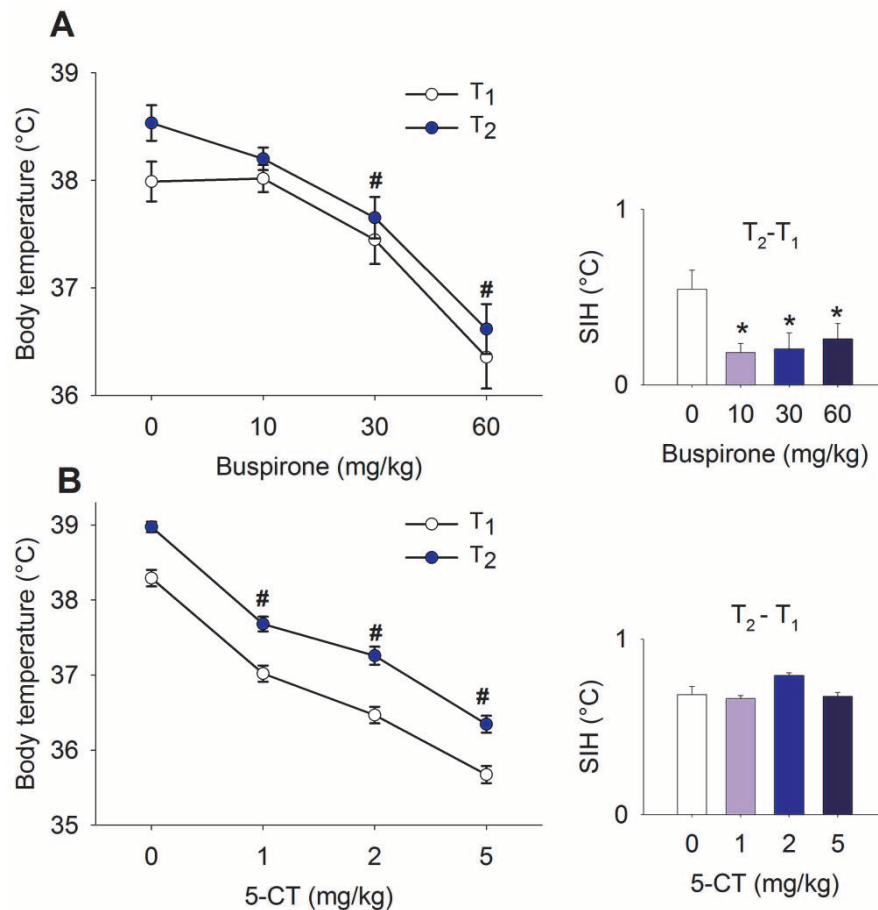
Drug	Species	Dose (mg/kg)	Route	Hypothermia	SIH ↓	Remarks	Ref.
Ipsapirone	Mouse (NMRI)	10-20	PO	N	N	G-SIH, 60 min	[110]
	Mouse (NMRI)	10-60	PO	N	Y		[109]
Flesinoxan	Mouse (NMRI)	0.3-3	PO	N	Y	G-SIH, 60 min	[110]
	Mouse (129Sv/Ola)	0.3-3	SC	Y	Y		[106]
	Mouse (129Sv/EvTac and C57Bl/6J)	0.3-3	SC	Y	Y		[157]
	Mouse (NMRI)	0.1-10	PO	Y	Y		[109]
	Mouse (129Sv)	0.3-3	SC	Y	Y		[130]
	Mouse (129Sv)	0.3-3	SC	Y	Y		[131]
	Mouse (129Sv, C57Bl/6J, Swiss)	0.3-3	IP	Y	Y	Three mouse strains compared	[15]
Buspirone	Mouse (Swiss)	10	IP	N	Y	G-SIH, 45 min	[111]
	Mouse (NMRI)	10-20	PO	N	Y	G-SIH, 60 min	[110]
	Mouse (NMRI)	10-60	PO	N	Y		[109]
	Mouse (129Sv, C57Bl/6J, Swiss)	1-4	IP	N	Y	Three strains compared	[115]
	Mouse (CD-1)	5-10	IP	Y	Y	G-SIH, 30 min	[112]
8-OH-DPAT	Mouse (Swiss)	2.5-10	SC	Y	Y	G-SIH, 30 min	[111]
	Mouse (NMRI)	1-10	SC	Y	Y		[109]
Flibanserin	Mouse (CD-1)	8-16	IP	N	Y	5-HT <sub>1A</sub> R agonist, G-SIH, 30 min	[112]
WAY100635	Mouse (NMRI)	0.001-10	SC	N	N	Hyperthermia at higher doses	[109]
WAY100635 + flesinoxan	Mouse (129Sv)	1.0 + 0.3-3	SC	N	N	WAY blocks flesinoxan effects on SIH	[131]
S-UH301	Mouse (NMRI)	1-30	SC	N	N	5-HT <sub>1A</sub> R antagonist	[109]
DU125530	Mouse (NMRI)	3-30	PO	N	N	5-HT <sub>1A</sub> R antagonist	[109]

ipsapirone did not affect the SIH response [110], although it was effective in the singly-housed paradigm though only at high doses (40 and 60 mg/kg) that were not tested in the group-housed mice [109]. Also, buspirone, registered as an anxiolytic in humans, dose-dependently decreases the SIH response in mice (Fig. 3A), confirming earlier SIH studies [110, 111, 113, 114]. This effect is absent in 5-HT<sub>1A</sub> receptor KO mice [115].

The hypothermic effects of 5-HT<sub>1A</sub> receptor agonists are in line with several preclinical and clinical studies [106, 116-118]. This effect is most probably induced through presynaptic 5-HT<sub>1A</sub> autoreceptors [119]. The hypothermic effects of 5-HT<sub>1A</sub> receptor agonists are probably mediated *via* the medullary rostral raphe pallidus, leading to cutaneous vasodilatation and decreased brown adipose tissue thermogenesis [120, 121], and common descending thermoregulatory pathways *via* the rostral raphe pallidus may explain the fact that 5-HT<sub>1A</sub> receptor agonists reduce lipopolysaccharide-induced fever [122, 123]. Chronic treatment with SSRIs attenuates 5-HT<sub>1A</sub> agonist-induced hypothermia in healthy subjects [124, 125] as well as in patients diagnosed with anxiety disorders and depression

[126-128], suggesting that the desensitization of the somatodendritic 5-HT<sub>1A</sub> receptor is involved in SSRI effects. A more selective 5-HT<sub>1A</sub> receptor agonist that preferentially acts on postsynaptic receptors would be of value as it may aid in distinguishing putative postsynaptic anxiolytic effects from presynaptic hypothermic processes [129]. 5-HT<sub>1A</sub> receptor knockout mice (1AKO) display an increased SIH response compared to wildtype mice after novel cage stress using telemetry but not after the manual rectal temperature measurement methods [130, 131]. This difference could be attributable to a differential stress responsivity in which a rectal temperature measurement - in contrast to novel cage stress - would not yield a different SIH response.

Altogether, 5-HT<sub>1A</sub> receptor ligands reduce the SIH response and the SIH paradigm is thus sensitive to detect the anxiolytic effects of 5-HT<sub>1A</sub> receptor agonists. In support, the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 is able to block the SIH-attenuating effects of 5-HT<sub>1A</sub> receptor agonists, while WAY-100635 (or other 5-HT<sub>1A</sub> receptor antagonists such as S-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin (S-UH-301) and 2-[4-[4-(7-Chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3-(2H)-one-1,1-



**Fig. (3).** Effects of 5-HT<sub>1A</sub> receptor agonist buspirone (0-60 mg/kg, IP) and 5-HT<sub>7</sub> receptor agonist 5-CT (0-5 mg/kg) on basal body temperature and the SIH response in 129Sv mice (n=10-12). \*: significant reduction of the SIH response (p<0.05); #: significant effect on basal body temperature (p<0.05). Error bars represent the S.E.M. Unpublished data.

dioxide (DU125530)) have no intrinsic effects on the SIH response of basal body temperature [109].

#### 4.2. Effects of Other 5-HT Receptor Drugs on the SIH Response (Table 2)

In contrast to the convincing SIH-reducing effects of 5-HT<sub>1A</sub> receptor agonists, other serotonergic drugs generally do not influence the SIH response [16]. Both the 5-HT<sub>1B</sub> receptor agonist eltopazine [110] as well as TFMPP [111] have no effect on the SIH response, and 5HT<sub>1B</sub> KO mice do not display altered SIH responses although an increased SIH response to novel cage stress as well as an increased basal body temperature have been reported [56]. Moreover, 5-HT<sub>2A/C</sub> receptor agonists and antagonists do not alter the SIH response. For example, no effects were found after administration of the 5-HT<sub>2A</sub> receptor agonist DOI [110], the 5-HT<sub>2C</sub> receptor agonist mCPP or the 5-HT<sub>2A/C</sub> receptor antagonists ketanserin or ritanserin [110, 111]. Although they do not affect the SIH response, 5-HT<sub>2</sub> receptor agonists increase and 5-HT<sub>2</sub> receptor antagonists decrease basal body temperature levels [110, 132, 133]. Interestingly, blockade of the 5-HT<sub>2</sub> receptor has been implicated in hypothermia during antipsychotic use [134]. Similar to 5-HT<sub>2A/C</sub> receptor ligands, 5-HT<sub>3</sub> receptor antagonists are ineffective in reducing the SIH response. Both DAU6215 [135] and ondansetron [109, 110] did not alter the SIH response.

The 5-HT<sub>7</sub> receptor agonist 5-carboxytryptamine (5-CT) induces hypothermia without affecting the SIH response (Fig. 3B), an effect that has also been reported in guinea-pigs [136] and mice [137]. 5-HT<sub>7</sub> receptor antagonists have been suggested to exert anxiolytic effects [97], although 5-HT<sub>7</sub> receptor agonist and antagonists do not alter the SIH response [15, 115].

#### 4.3. Effects of Acute and Chronic Antidepressant Treatment on the SIH Response (Tables 3 and 4)

Chronic treatment with tricyclic antidepressants or SSRIs remains the mainstay in the management of major depressive disorder and anxiety disorders. SSRIs selectively bind to the SERT and inhibit 5-HT reuptake which results in an increase of extracellular 5-HT. The majority of TCAs act primarily as serotonin-norepinephrine reuptake inhibitors (SNRIs) by blocking the SERT and the norepinephrine transporter (NET), resulting in an increase of the extracellular concentrations of these neurotransmitters, and thereby enhances their neurotransmission [138, 139]. An acute single bolus of a SSRI or TCA does not alter the SIH response as has been reported for fluvoxamine, escitalopram, desipramine, imipramine, fluoxetine, and amitriptyline [109, 110, 113]. In general, some serotonergic antidepressants induce hypothermia without affecting the SIH response. Fig. (4) shows the typical hypothermic (with no effect on the SIH

**Table 2. Effects of Other 5-HT Receptor Ligands on Basal Body Temperature (T<sub>1</sub>, Hypothermia) and the Stress-Induced Hyperthermia (SIH) Response. G-SIH: Group-House SIH Paradigm, Including the Injection-Stressor Interval (Minutes). PO: Oral, IP: Intraperitoneal**

Drug	Species	Dose (mg/kg)	Route	Hypothermia	SIH ↓	Remarks	Ref.
TFMPP	Mouse (Swiss)	5-20	IP	N	N	5-HT <sub>1A/B</sub> agonist, G-SIH, 45 min	[111]
	Mouse (NMRI)	3-30	PO	Y	Y	5-HT <sub>1A/B</sub> agonist	[109]
RU24969	Mouse (NMRI)	3-30	PO	Y	Y	5-HT <sub>1A/B</sub> agonist	[109]
Aniprotiline	Mouse (NMRI)	1-10	PO	Y	N	5-HT <sub>1B</sub> agonist	[109]
DOI	Mouse (NMRI)	0.3-3	PO	Hyperthermia	Y, due to hyperthermia	5-HT <sub>2A/C</sub> agonist, G-SIH	[110]
	Mouse (NMRI)	0.3-10	PO	N	N	5-HT <sub>2A/C</sub> agonist	[109]
mCPP	Mouse (Swiss)	2.5-5	IP	N	N	5-HT <sub>2c</sub> agonist, G-SIH, 45 min	[111]
	Mouse (NMRI)	1-10	PO	N	N	5-HT <sub>2c</sub> agonist, G-SIH, 60 min	[110]
	Mouse (NMRI)	3-30	PO	N	N	5-HT <sub>2c</sub> agonist	[109]
	Mouse (129Sv, C57Bl/6J, Swiss)	1-10	IP	Y/N	N	5-HT <sub>2c</sub> agonist	This review
Ketanserin	Mouse (Swiss)	0.1-0.2	IP	N	N	5-HT <sub>2A/2C</sub> antagonist, G-SIH, 60 min	[111]
	Mouse (NMRI)	1-10	PO	N	N	5-HT <sub>2A/2C</sub> antagonist, G-SIH, 60 min	[110]
	Mouse (NMRI)	1-10	PO	Y	N	5-HT <sub>2A/2C</sub> antagonist	[109]
Ritanserin	Mouse (Swiss)	0.1-0.2	IP	N	N	5-HT <sub>2A/2C</sub> antagonist, G-SIH, 60 min	[111]
	Mouse (NMRI)	1-30	PO	Y	Y	5-HT <sub>2A/2C</sub> antagonist	[109]
DAU6215	Mouse (CD-1)	1-100 µg/kg	IP	N	N	5-HT <sub>3</sub> antagonist. G-SIH, 45 min	[135]
Ondansetron	Mouse (Swiss)	0.1-100 µg/kg	IP	N	N	5-HT <sub>3R</sub> antagonist, G-SIH	[111]
	Mouse (NMRI)	0.1-100 µg/kg	IP	N	N	5-HT <sub>3R</sub> antagonist, G-SIH	[110]
	Mouse (CD-1)	1-100 µg/kg	IP	N	N	5-HT <sub>3R</sub> antagonist, G-SIH 45 min	[135]
	Mouse (NMRI)	0.001-1	IP	N	N	5-HT <sub>3R</sub> antagonist	[109]
MCPB	Mouse (NMRI)	1-10	PO	N	N	5-HT <sub>3R</sub> agonist	[109]
Eltoprazine	Mouse (NMRI)	1-10 mg/kg	IP	Y/N (U shaped effect)	N	5-HT <sub>1A/B/R</sub> agonist, G-SIH	[110]
LY53857	Mouse (Swiss)	1.5-3	IP	N	N	5-HT <sub>2R</sub> antagonist, G-SIH	[111]
5-CT	Mouse (129Sv, C57Bl/6J, Swiss)	0.5-2	PO	Y	N	5-HT <sub>7R</sub> agonist	This review
	Mouse (129Sv, C57Bl/6J, Swiss)	0.5-2	IP	Y	N		[115]
	Mouse (C57Bl/6J)	1-5	IP	Y	N		[15]

response) of acute escitalopram administration in Wistar rats (unpublished data). Besides escitalopram, fluoxetine, desipramine and amitriptyline have been shown to cause hypothermia without altering the SIH response (Table 3). In contrast, hypothermic effects of acute SSRI, TCA or serotonin modulating drugs may also be absent, for example with fluvoxamine, clorgyline, clomipramine imipramine and tianeptine. The apparent differences are unclear and do not seem attributable to the pharmacological profile of the

compounds. Alternatively, the dose and/or strain may influence the hypothermic effects of antidepressants.

In contrast to acute data, there is a paucity of data on the effects of chronic antidepressant treatment on the SIH response. Because SSRIs are effective in the treatment of anxiety disorders, chronic SSRI treatment would be expected to alter the SIH response. However, the evidence so far has been inconclusive, and the majority of the SIH studies using chronic administration do find no effect on the SIH response. In one study, chronic but not acute fluoxetine treatment

**Table 3. Effects of Acute Administration of SSRIs, TCAs and MAOIs on Basal Body Temperature (T<sub>1</sub>, Hypothermia) and the Stress-Induced Hyperthermia (SIH) Response. G-SIH: Group-House SIH Paradigm, Including the Injection-Stressor Interval (Minutes). PO: Oral, IP: Intraperitoneal, SC: Subcutaneous**

Drug	Species	Dose (mg/kg)	Route	Hypothermia	SIH ↓	Remarks	Ref.
Fluvoxamine	Mouse (NMRI)	3-30	PO	N	Y, due to hyperthermia	G-SIH, 60 min	[110]
	Mouse (NMRI)	3-30	PO	N	N		[109]
Escitalopram	Mouse (C57Bl/6J)	2-10	IP	Y	N		[15]
Fluoxetine	Mouse (Swiss)	10-20	IP	Y	N	G-SIH	[113]
	Mouse (NMRI)	3-30	PO	N	N		[109]
Tranylcypromine	Mouse (Swiss)	5-10	IP	Y	N	MAO-I, G-SIH	[113]
Clorgyline	Mouse (NMRI)	10-30	PO	N	N	MAO <sub>A</sub> -I	[109]
Desipramine	Mouse (Swiss)	15-30	SC	Y	N	G-SIH, 45 min	[113]
	Mouse (NMRI)	3-30	PO	Y	N	G-SIH, 60 min	[110]
	Mouse (NMRI)	3-30	PO	N	N		[109]
Clomipramine	Mouse (NMRI)	3-30	PO	N	N	G-SIH, 60 min	[110]
	Mouse (NMRI)	3-30	PO	N	N		[109]
Amitriptyline	Mouse (Swiss)	10	IP	Y	N	G-SIH	[113]
	Mouse (NMRI)	3-30	PO	Y	N	G-SIH	[110]
	Mouse (NMRI)	3-30	PO	Y	N		[109]
Imipramine	Mouse (Swiss)	15-30	IP	N	N	G-SIH	[14]
	Mouse (NMRI)	3-30	PO	N	N	G-SIH	[110]
	Mouse (NMRI)	10-30	PO	N	N		[109]
Tianeptine	Mouse (NMRI)	3-30	PO	N	Y, due to hyperthermia	G-SIH, serotonin enhancer	[110]
	Mouse (NMRI)	3-30	PO	N	Y, due to hyperthermia	serotonin enhancer	[109]
PCPA	Mouse (Swiss)	75-150	IP	N	N	5-HT depletor Injected 72,48,24 before test, G-SIH	[111]
5,7 DHT	Mouse (Swiss)	200 ug	ICV	N	N	9 days before test, G-SIH, reverses buspirone induced SIH reduction	[111]
D,l-fenfluramine	Mouse (NMRI)	3-30	PO	N	N	5-HT releaser, G-SIH	[110]
	Mouse (NMRI)	3-30	IP	N	Y	5-HT releaser	[109]

reduced the SIH response in rats and mice [140]. However, conflicting data exist whether chronic antidepressant treatment is able to reduce the SIH response, since Roche and colleagues did not find any attenuation in the SIH after chronic fluoxetine treatment in rats [141]. Also, no effects of chronic SSRI treatment (fluoxetine, imipramine and amitriptyline) on the SIH response were found in mice (Table 4). Thus, the SIH paradigm may be insensitive to detect the anxiolytic effects of chronic serotonergic antidepressants. The discrepancy between clinical drug efficacy vs inefficacy in the SIH paradigm may at least be partially explained by the fact that the SIH response generally constitutes a normal and healthy stress response. The drugs that have been found to reduce the SIH response (e.g. benzodiazepines), acutely do so irrespective of the healthy or pathological status of an individual. This way, one may argue that chronic exposure to SSRIs would only alter

the SIH response under pathological conditions. However, except the SERT knockout rat [142], an altered SIH response is not a common finding in genetically modified animals with increased anxiety levels, such as 1AKO mice.

SIH responsiveness has also been assessed in SERT knockout (SERT<sup>-/-</sup>) mice and rats [142, 143]. The SERT<sup>-/-</sup> rat or mice has been developed as an animal model of depression, and, comparable to humans treated with SSRIs, these rats [142, 144] and mice [145-147] possess reduced 5-HT<sub>1A</sub> receptor reactivity. In SERT<sup>-/-</sup> mice, no differences in SIH responsiveness were found [143]. In contrast, SERT<sup>-/-</sup> rats displayed a decreased SIH response after a saline injection, although novel cage stress elicited a similar SIH response in SERT<sup>-/-</sup> rats compared to wildtype animals [142]. Thus, differences in the SIH response in SERT<sup>-/-</sup> rats appear with decreased intense stressor intensity. In support, a differential SIH reactivity that depends on stressor intensity



**Table 4. Effects of Chronic SSRI Treatment on Basal Body Temperature ( $T_1$ , Hypothermia) and the Stress-Induced Hyperthermia (SIH) Response. G-SIH: Group-House SIH Paradigm, Including the Injection-Stressor Interval (Minutes). PO: Oral, IP: Intraperitoneal, SC: Subcutaneous**

Drug	Species	Dose (mg/kg)	Route	Hypothermia	SIH ↓	Remarks	Ref.
Fluoxetine	Mouse (Swiss)	10	IP	N	N	7, 14 and 21 day period, G-SIH	[113]
Imipramine	Mouse (Swiss)	10	IP	N	N	7, 14 and 21 day period, G-SIH	[113]
Amitriptyline	Mouse (Swiss)	10	IP	N	N	7, 14 and 21 day period, G-SIH	[113]
Fluoxetine	Rat (Sprague-dawley)	10	SC	N	N	35 days, SIH restoration in OBX rats	[141]
Fluoxetine	Mouse (SW) and rat (CD-1)	15 and 10	PO	N	YW	21 days	[140]

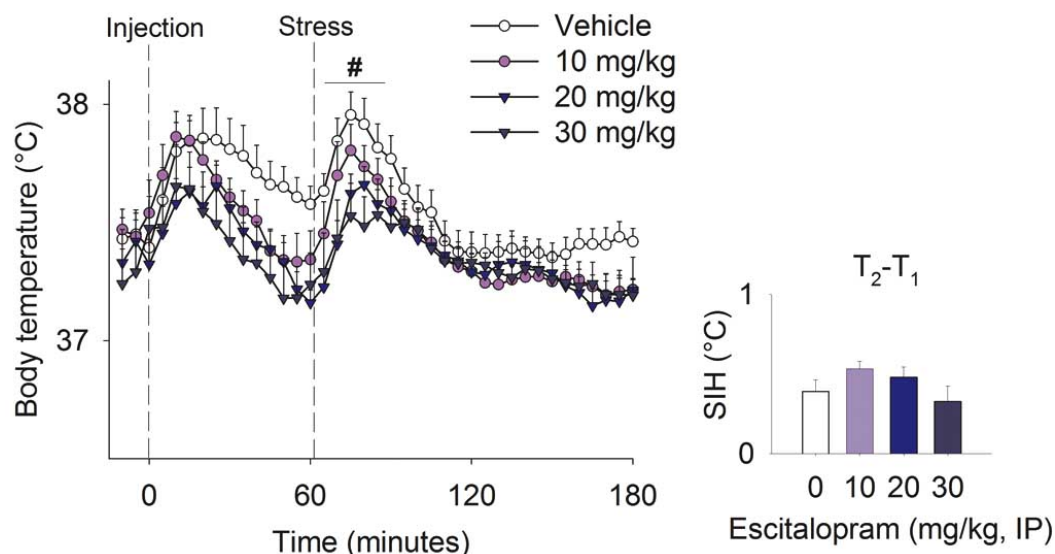
was also found in olfactory bulbectomized animals which possess altered 5-HT functionality [148]. Interestingly, the 5-HT<sub>1A</sub> receptor agonist flesinoxan did not result in the regular hypothermic effects in SERT<sup>-/-</sup> rats, whereas the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 increased the body temperature in SERT<sup>-/-</sup> rats compared to SERT<sup>+/+</sup> rats (Fig. 5). So far, it is unclear whether this WAY-100635-induced hyperthermia in SERT<sup>-/-</sup> rats is due to an altered thermoregulation or altered anxiety-related circuitry.

#### 4.4. Effects of Serotonin Release or Depletion on the SIH Response

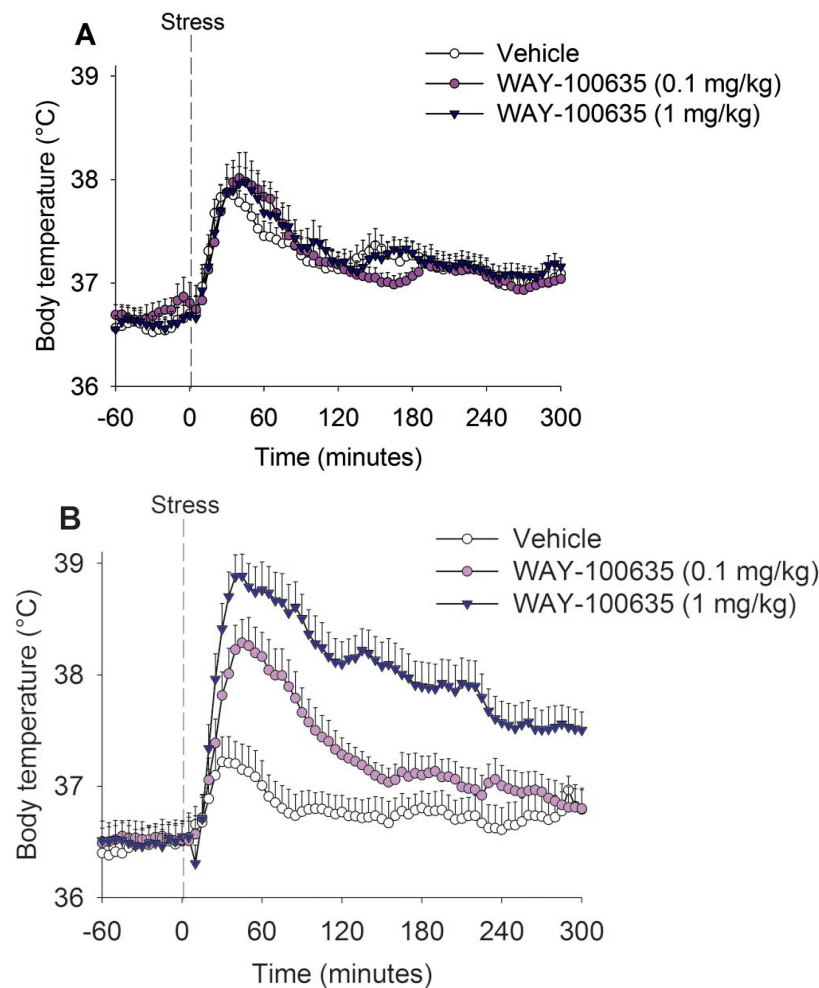
Fenfluramine is a 5-HT releaser that disrupts vesicular 5-HT storage as well as binds to SERT, thereby increasing extracellular 5-HT levels. Fenfluramine was found to be ineffective in lowering the SIH response [110]. Moreover, the selective serotonin reuptake enhancer tianeptine did not affect the SIH response, even though it lowered extracellular 5-HT levels [110]. As tryptophan is the precursor of serotonin, diet depletion of l-tryptophan results in lower 5-HT levels in the CNS which has resulted in mood depression in at-risk individuals [149, 150]. So far, acute tryptophan depletion has not been studied in the SIH paradigm, even though depletion resulted in core body temperature increases in rats (Fig. 6).

#### 4.5. Effects of Serotonin-Mediated Alterations of Other Neurotransmitter Systems

In addition to direct serotonergic effects on the SIH response, a number of studies addressed the hypothesis that serotonin may affect other neurotransmitter systems in altering the SIH response. IAKO mice on a Swiss-Webster (SW) background possess reduced benzodiazepine sensitivity, and this serotonin-induced benzodiazepine insensitivity in mice on the SW but not the C57Bl6/J or 129Sv background was confirmed using the SIH paradigm [56, 115, 131, 151, 152]. IAKO mice on a SW background were insensitive to the GABA<sub>A</sub> receptor agonists diazepam and L838,417, while SIH reduction was apparent in wildtype mice [115]. Moreover, the inability of benzodiazepines to reduce the SIH response in 5-HT<sub>1A</sub> receptor KO mice was replicated in wildtype mice after the pharmacological 5-HT<sub>1A</sub> receptor blockade with WAY-100635 during the early postnatal period [153]. This way, long-lasting benzodiazepine insensitivity was found in adolescent as well as adult mice using the SIH paradigm. WAY-100635-treated mice also showed increased cortical GABA<sub>A</sub>  $\alpha_1$  and  $\alpha_3$  subunit levels and increased hippocampal GABA<sub>A</sub>  $\alpha_2$  subunit levels. Thus, early-life disruption of the 5-HT system may affect benzodiazepine sensitivity in later life. Recently, the SIH-reducing effects of group II metabotropic glutamate



**Fig. (4).** Acute administration of the SSRI escitalopram (0-30 mg/kg, IP) does not affect the SIH response in Wistar rats (n=11), but it lowers basal body temperature. #: overall drug effect on basal body temperature (p<0.05). Error bars represent the S.E.M. Unpublished data.



**Fig. (5).** Stress-induced hyperthermia response after administration of the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (0.1 mg/kg, IP) in SERT<sup>+/+</sup> rats (**A**, n=8) and in SERT<sup>-/-</sup> rats (**B**, n=7-8). Values are mean  $\pm$  SEM. No drug effect was found. In SERT<sup>+/+</sup> rats, no differences in WAY-100635 response were found. In contrast, significant differences were found between WAY-100635 doses in SERT<sup>-/-</sup> rats compared to vehicle treatment ( $F_{(144,1728)}=10.764$ ;  $p<0.001$ ) [142].

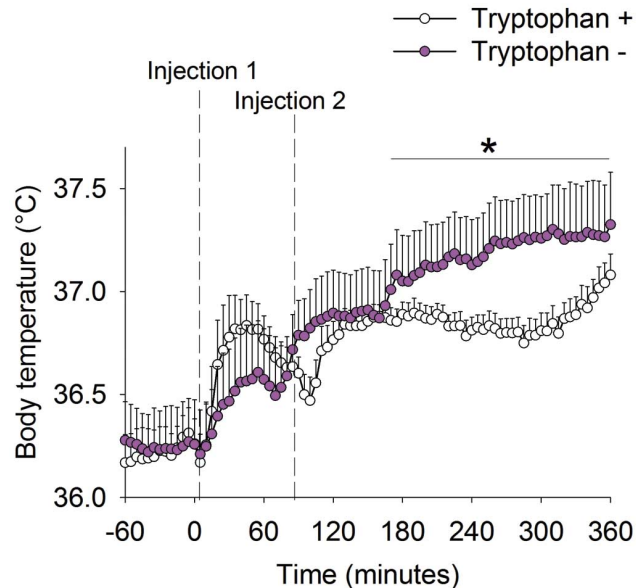
(mGlu) 2/3 receptor agonists MGS0039 and LY341495 could be reversed after co-administration of the 5-HT<sub>1A</sub> receptor antagonist WAY-100635, suggesting that glutamatergic drugs act *via* the serotonin system to exert its anxiolytic effects [154]. Moreover, the 5-HT<sub>1A</sub> receptor appeared to modulate the SIH-reducing effects of benzodiazepines *via* the GABA<sub>A</sub> receptor  $\alpha_3$  subunit [158]. Altogether, these data indicate that the SIH paradigm may be employed to study the acute and chronic interactions of different neurotransmitter systems on the autonomic stress response.

## 5. CONCLUSION

Preclinical studies indicate that disruption of the serotonergic system from the early-life to adult period may influence stress responsivity, and a normal functionality of the serotonergic system is essential to prevent affective disorders. The structurally, anatomically and functionally complex serotonin system is involved in stress-related behavior and vulnerability to develop psychiatric disorders. So far, certain serotonergic ligands have been shown to differentially affect the autonomic SIH response. Activation of 5-HT<sub>1A</sub> receptors convincingly reduces the SIH response,

confirming the anxiolytic potential of this receptor class. In contrast, modulation of other 5-HT receptor types including 5-HT<sub>1B</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptors does generally not influence the SIH response. It is unclear why these ligands are anxiolytic in some other anxiety paradigms yet do not affect the SIH response. However, no selective drugs that modulate these receptor types have been registered for the clinical treatment of stress-related disorders as anxiety or depression. Also, putative anxiogenic drugs do not increase the autonomic SIH response, suggesting that increased anxiety levels are not automatically accompanied by higher autonomic stress responsivity. Although anxiogenic compounds are considered to heighten the stress response, the effects depend on the model and specific compound used. In support, tachycardia in anxious people depends on the situation and diagnosis, and a more avoiding personality is associated with reduced heart rate responses [155]. Moreover, patients with panic disorder (PD) display comparable physiological responses to healthy controls, even though they experience more frequent distress, suggesting that the perception of stress in anxiety disorders is not accompanied by heightened autonomic responses [156]. Together, these data suggest that increased subjective stress

levels due to (serotonergic) anxiogenic drugs may not necessarily be accompanied by increased autonomic stress responsivity.



**Fig. (6).** The effect of acute tryptophan depletion on core body temperature in Wistar rats. Rats were treated with a protein-carbohydrate mixture containing TRP (TRP+ group, 0.30% TRP of the total protein) or lacking TRP (TRP- group). The rats received two TRP doses (PO, 10 ml/kg) with a 90-minute interval. Significant differences were found between TRP+ and TRP-treatment ( $F_{(84,1260)}=3.418$ ;  $p<0.001$ ). Unpublished data.

The 5-HT system is regulated via SERT modulation, and SERT dysfunction has been implicated in anxiety-related behavior. Acute and chronic administration of drugs affecting the SERT (which include TCAs and SSRIs) are generally ineffective in reducing the SIH response. The SIH paradigm is sensitive to acute anxiolytic effects of different drug classes that reduce the stress response irrespective of an individual's mental health. Hypothetically, chronic SSRIs treatment could affect the SIH response under pathological conditions. If so, it would be necessary to establish the SIH response in patients suffering from a stress-related disorder. If an increased, attenuated, or inappropriately activated SIH response is present in stress-related disorders, it would be interesting to follow the effects of chronic SSRI treatment on the autonomic SIH response. In addition to direct serotonergic effects on the SIH response, there are indications that SIH-reducing effects of other neurotransmitter systems including the GABA<sub>A</sub> and glutamate receptor system may be at least partially mediated through the 5-HT system. Thus, the SIH paradigm may be employed to study the acute and chronic interactions of different neurotransmitter systems on the acute stress response, including putative serotonergic interactions.

In conclusion, the present review shows that serotonergic drugs that have been found to reduce the SIH response acutely do so irrespective of the healthy or pathological status of an individual, and that the SIH paradigm is generally insensitive to detect the anxiolytic effects of chronic serotonergic antidepressants in rodents.

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