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Serotonin and serotonin transporters in the adrenal medulla: a potential hub for modulation of the sympathetic stress response

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Abstract

Serotonin (5-HT) is an important neurotransmitter in the central nervous system where it modulates circuits involved in mood, cognition, movement, arousal, and autonomic function. The 5-HT transporter (SERT; *SLC6A4*) is a key regulator of 5-HT signaling, and genetic variations in SERT are associated with various disorders including depression, anxiety, and autism. This review focuses on the role of SERT in the sympathetic nervous system. Autonomic / sympathetic dysfunction is evident in patients with depression, anxiety, and other diseases linked to serotonergic signaling. Experimentally, loss of SERT function (SERT knockout mice or chronic pharmacological block) has been reported to augment the sympathetic stress response. Alterations to serotonergic signaling in the CNS and thus central drive to the peripheral sympathetic nervous system are presumed to underlie this augmentation. Although less widely recognized, SERT is robustly expressed in chromaffin cells of the adrenal medulla, the neuroendocrine arm of the sympathetic nervous system. Adrenal chromaffin cells do not synthesize 5-HT but accumulate small amounts by SERT-mediated uptake. Recent evidence demonstrated that 5-HT_{1A} receptors inhibit catecholamine secretion from adrenal chromaffin cells via an atypical mechanism that does not involve modulation of cellular excitability or voltage-gated Ca^{2+} channels. This raises the possibility that the adrenal medulla is a previously unrecognized peripheral hub for serotonergic control of the sympathetic stress response. As a framework for future investigation, a model is proposed in which stress-evoked adrenal catecholamine secretion is fine-tuned by SERT-modulated autocrine 5-HT signaling.

Graphical abstract

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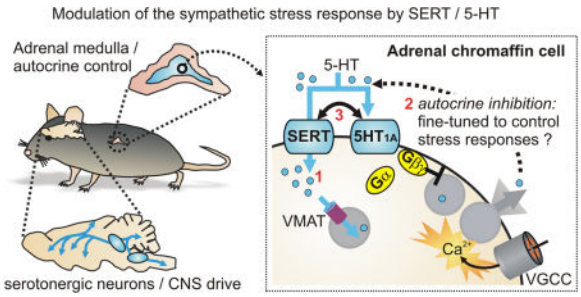

Keywords

serotonin transporter; adrenal chromaffin cell; sympathetic nervous system; calcium channel; exocytosis; catecholamine; 5-HT receptor

Introduction

Serotonin (5-HT) is an important neurotransmitter and neuromodulator in the central nervous system and controls a variety of physiological processes including mood, cognition, movement, arousal, and autonomic functions. The 5-HT transporter (SERT; *SLC6A4*) is a key regulator of 5-HT signaling; SERT-mediated reuptake by serotonergic neurons is one of the main mechanisms for clearing extracellular 5-HT after release, thereby terminating signaling and recycling 5-HT for subsequent rounds of release. Genetic variations that alter the expression or activity of SERT have been linked with depression, anxiety disorders, obsessive compulsive disorder, and autism [1-3]. Moreover, SERT is a target for psychostimulant drugs (e.g. cocaine and MDMA / ecstasy) and widely prescribed antidepressants (e.g. selective serotonin reuptake inhibitors - SSRIs) [4].

In this article we focus on the role of SERT / 5-HT signaling in the peripheral sympathetic nervous system. Autonomic dysfunction, including an aberrant sympathoadrenal stress response, is evident in depression, anxiety, and other diseases linked to serotonergic signaling[5-12]. Experimental evidence has also implicated SERT in the sympathetic stress response; SSRIs enhance the counterregulatory release of epinephrine in response to hypoglycemic stress in rodents [13] and humans [14, 15]. In other studies, restraint stress evoked an exaggerated increase in plasma epinephrine in mice with constitutive (global) knockout of SERT (SERT$^{-/-}$ mice) [16-18]. The underlying mechanism is presumed to involve altered SERT / 5-HT signaling in the CNS which increases central drive to the peripheral sympathetic nervous system. Although not widely recognized, SERT is robustly expressed in adrenal chromaffin cells, the neuroendocrine arm of the sympathetic nervous system [19] and in a subset (~30%) of neurons in the sympathetic ganglia [20]. Here we outline the pathways by which SERT / 5-HT signaling in the CNS might modulate central drive to the sympathetic nervous system. We discuss the potential role(s) of SERT in the sympathoadrenal system, and the possibility that the adrenal medulla is a peripheral hub for serotonergic control of the sympathetic stress response. A working model is also outlined and intended to provide a framework for future experiments to test this central hypothesis.

Overview of the sympathoadrenal system and potential sites of serotonergic regulation in the CNS

Serotonergic neurons in the CNS are clustered in the raphe nuclei of the brainstem and, although relatively few in number, project extensively throughout the brain and spinal cord [21-25] (Figure 1A). The various raphe nuclei are interconnected and their activity is modulated by input from multiple other brain regions. In general, the rostral midbrain raphe nuclei send ascending projections to brain regions including cerebral cortex, hippocampus, hypothalamus, thalamus, basal ganglia and amygdala whereas the caudal raphe nuclei (raphe pallidus, raphe magnus and raphe obscurus) send afferents to the cerebellum and descending projections to the spinal cord. Both the rostral and caudal raphe nuclei innervate brainstem neurons that regulate the sympathetic stress response, including those in the rostral ventrolateral medulla (RVLM). The RVLM contains a heterogeneous population of neurons (predominantly glutamatergic and catecholaminergic) that provide the primary excitatory drive to preganglionic sympathetic neurons in the intermediolateral columns of the thoracic spinal cord. The preganglionic neurons also receive direct input from the rostral ventromedial medulla, A5 noradrenergic cell group, paraventricular nucleus of the hypothalamus, and the caudal raphe nuclei [25-30] (Figure 1B). Spinal preganglionic neurons project to peripheral sympathetic ganglia where they release acetylcholine (ACh) to excite postganglionic sympathetic neurons. In turn, the postganglionic neurons innervate specific target organs, releasing norepinephrine (NE) and other co-transmitters to exert local control of these effectors (e.g. heart, vascular smooth muscle, immune system, etc.) (Figure 1C). Preganglionic sympathetic neurons also project via the splanchnic nerve to chromaffin cells in the adrenal medulla. Derived from the neural crest, adrenal chromaffin cells behave much like postganglionic sympathetic neurons and comprise the neuroendocrine arm of the sympathetic nervous system. Upon stimulation they release catecholamines and neuropeptides into the bloodstream to exert widespread effects on the cardiovascular, endocrine, immune, and nervous systems [31-36] (Figure 1C). Unlike sympathetic neurons, most (~80%) adrenal chromaffin cells express phenylethanolamine-N-methyltransferase (PNMT), the enzyme that converts norepinephrine into epinephrine (Epi). These “adrenergic” chromaffin cells release primarily epinephrine while the “noradrenergic” chromaffin cells lacking PNMT release norepinephrine. The vast majority of plasma epinephrine (>95%) is due to secretion from adrenal chromaffin cells whereas plasma norepinephrine is partly due to secretion from chromaffin cells (10-30%) but mostly due to overflow from postganglionic nerve terminals [37, 38]. Together, the sympathoneural and sympathoadrenal arms of the sympathetic nervous system coordinate the physiological and/or pathophysiological responses to environmental, metabolic, and emotional/psychological stressors. Distinct subsets of RVLM neurons can be activated in a stressor specific manner resulting in preferential recruitment of the sympathoneural (e.g. cold stress) or sympathoadrenal responses (hypoglycemia) [27, 39-42]. Additionally, evidence suggests distinct preganglionic innervation and stressor specific activation of adrenergic and noradrenergic chromaffin cells [43-46].

As noted above, SERT is expressed on the axons and synaptic terminals of serotonergic neurons that project widely throughout the CNS. Consequently, genetic loss or

pharmacological block of SERT at multiple sites within the CNS could ultimately alter the excitability (increase or decrease) of sympathetic preganglionic neurons. For example, 5-HT can act directly within the RVLM to inhibit the sympathetic premotor neurons, likely via 5-HT_{1A} receptors, although excitation via 5-HT_2 receptors has been reported [47-51]. Altered SERT function could also have less direct effects on the excitability of RVLM neurons by modulating input from other brain regions. In the spinal cord, immunocytochemical and retrograde labeling studies show that SERT is expressed on presynaptic boutons innervating the dendrites and soma of preganglionic sympathetic neurons, including those that innervate the adrenal gland [52-55]. These serotonergic projections are thought to arise directly from the caudal raphe nuclei, although there is a small population of spinal serotonergic neurons [56, 57]. The density of serotonergic innervation is not uniform along the rostral-caudal axis of the thoracolumbar spinal cord, which may result in differential modulation of specific sympathetic responses by SERT/5-HT [25, 55, 58]. Although inhibition has been reported [59, 60], 5-HT is generally thought to excite preganglionic sympathetic neurons in the spinal cord by 5-HT_2 receptor dependent mechanisms or through 5-HT_{1A} receptors that produce a disinhibition of spinal interneuron input to the sympathetic neurons [59-64].

To summarize, SERT / 5-HT signaling in multiple regions of the brain and spinal cord can potentially increase or decrease central drive to the peripheral sympathetic nervous system. The overall effect will vary depending on the neuronal pathways recruited by the stressor and the serotonergic innervation and receptor expression in those respective pathways. For example, loss of SERT function enhanced the increase in plasma epinephrine, but not norepinephrine, evoked by restraint stress or hypoglycemia [13-16]. This could be explained if loss of central SERT selectively altered the activity of neurons innervating the adrenergic population of chromaffin cells. Alternatively, as discussed below, SERT could act locally within the adrenal gland to modulate epinephrine secretion.

Multiple roles for SERT in sympathoadrenal chromaffin cells

In addition to mechanisms that modulate drive from the CNS, the sympathetic nervous system can also be regulated at peripheral sites including plasticity in ganglionic synaptic transmission and autocrine regulation of norepinephrine secretion in target tissues by presynaptic G protein coupled receptors (GPCRs) [65-70]. Similarly, catecholamine secretion can be regulated locally within the adrenal medulla by plasticity at the splanchnic-chromaffin cell synapse [71] and by a variety of GPCRs on chromaffin cells that respond to autocrine, paracrine, endocrine and neuronal transmitters [72-74]. Autocrine / paracrine control of adrenal catecholamine secretion is physiologically significant *in vivo*. For example, elevated sympathetic tone and plasma catecholamines are hallmarks of heart failure [75]. In a rodent model of heart failure, this elevation involved upregulation of GRK2 in the adrenal gland and consequent loss of GPCR-mediated autocrine inhibition [76, 77]. Correcting this imbalance using *in vivo* viral transduction to inhibit GRK2 selectively in adrenal chromaffin cells reduced plasma catecholamines and improved heart failure symptoms.

Although not widely recognized, SERT is highly expressed and co-localizes with PNMT in chromaffin cells of the adrenal medulla [19]. This raises the possibility that SERT / 5-HT signaling might act locally within the adrenal gland to control the sympathetic stress

response. Consistent with that, it is becoming clear that SERT has several distinct effects on chromaffin cell function and might coordinate serotonergic regulation of catecholamine exocytosis via 5-HT_{1A} receptors (Figure 2). One role for SERT is to accumulate 5-HT into the adrenergic chromaffin cells, which lack the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase [78-82]. The amount of 5-HT in chromaffin cells is ≈ 750 fold lower than epinephrine, but this is still an appreciable amount considering that vesicular catecholamine concentrations approach 0.5-1 molar [83, 84]. Moreover, when 5-HT was infused into rodents, the adrenal medulla displayed prominent SERT-mediated accumulation of exogenous 5-HT [82]. Conversely, the 5-HT content of adrenal glands isolated from $SERT^{-/-}$ mice or rats was dramatically reduced (≈ 80%) compared to wild-type controls, with no change in the catecholamine content [16, 82, 85]. A likely source of 5-HT is the circulation; the adrenal gland is highly vascularized and blood contains large amounts of 5-HT, although most of this is sequestered into platelets by SERT-mediated uptake [86, 87]. Other possible sources include mast cells in the adrenal cortex which are capable of synthesizing 5-HT [88], or neuronal input. Preganglionic splanchnic nerve terminals do not contain 5-HT, but sensory and vagal neuron terminals are also present in the adrenal medulla [89], and some of these might contain 5-HT [90]. Following SERT-mediated uptake, some of the 5-HT is likely transported into secretory vesicles by vesicular monoamine transporters and the balance subject to metabolism by monoamine oxidase in the cytoplasm. Thus 5-HT would then be available for exocytosis along with catecholamines and neuropeptides. Given the small amounts of 5-HT present in the adrenal gland and the efficient uptake of 5-HT by circulating platelets, we speculate that sufficient concentrations of 5-HT for receptor action are only achieved locally within the adrenal gland where the indoleamine would act as an autocrine / paracrine agent.

SERT modulates 5-HT_{1A} receptor-mediated inhibition of catecholamine secretion

Consistent with a local signaling role for 5-HT in adrenal chromaffin cells, our recent work revealed that catecholamine secretion can be inhibited by 5-HT_{1A} receptors [85]. In these studies, we monitored catecholamine release from mouse chromaffin cells *ex vivo* using carbon fiber amperometry, which enables the detection of quantal vesicular release events. We found that exocytosis was inhibited by 5-HT_{1A} receptors and that SERT-mediated uptake opposed the ability of 5-HT to recruit this pathway. The inhibition by 5-HT_{1A} receptors resulted in fewer vesicles undergoing exocytosis, whereas the amount (quantal size) and kinetics of transmitter release from individual vesicular fusion events were not changed. Voltage-gated Ca^{2+} channels play a pivotal role in stimulus-secretion coupling and are an important target for regulation by GPCRs at presynaptic terminals and adrenal chromaffin cells [65, 66, 91-93]. In the CNS, 5-HT_{1A} receptors inhibit voltage-gated Ca^{2+}channel currents (I_{Ca}) and somatodendritic 5-HT_{1A} receptors can also modulate cellular excitability through K^+ channel activation [94-99]. Surprisingly, although the inhibition of catecholamine secretion by 5-HT_{1A} receptors in adrenal chromaffin cells involved $G_{i/o}$-type G proteins and Gβγ signaling, it did not involve modulation of Ca^{2+} channels, K^+ channels, or changes in intracellular [Ca^{2+}] [85]. Previous reports have shown that Gβγ can inhibit secretion independently from Ca^{2+} channel regulation in chromaffin cells [100, 101] and neurons [102-104].

The "downstream" molecular target(s) of Gβγ in chromaffin cells remain to be defined, but one possible mechanism involves binding of Gβγ directly to SNARE proteins which comprise the core exocytotic fusion machinery [105]. Gβγ can compete with Ca^{2+}-bound synaptotagmin-1 for binding to the C-terminus of SNAP25, which might disrupt the triggering of exocytosis [106, 107]. Another proposed target of Gβγ is dynamin [101] which can modulate both exocytosis and endocytosis in chromaffin cells [108-110].

The 5-HT mediated autocrine inhibition outlined above for adrenal chromaffin cells has some parallels with serotonergic signaling in the CNS. In the CNS, $5\text{-}HT_{1A}$ receptors are predominantly expressed in the somatodendritic compartment of neurons whereas $5\text{-}HT_{1B}$ receptors are predominantly expressed at presynaptic sites [111-113]. Locally released 5-HT can control serotonergic neuron excitability through somatodendritic $5\text{-}HT_{1A}$ receptors which activate potassium channels [97-99], whilst $5\text{-}HT_{1B}$ receptors are thought to mediate direct presynaptic autocrine inhibition of 5-HT release and heterosynaptic inhibition of glutamatergic transmission. The mechanisms underlying $5\text{-}HT_{1B}$ receptor-mediated inhibition of neurotransmitter release include the classical modulation of presynaptic voltage-gated Ca^{2+}channels [114], and Ca^{2+}channel independent effects perhaps involving Gβγ-mediated modulation of the SNARE machinery [104]. Also of note, $5\text{-}HT_{1B}$ receptors can modulate SERT function in the CNS [115, 116].

SERT / 5-HT might modulate the late steps of exocytosis

SERT might have additional effects in adrenal chromaffin cells beyond those discussed above. For example, the amount of transmitter released by unitary vesicular fusion events (i.e. quantal size) was decreased by ≈35 % in $SERT^{-/-}$ cells compared to wild-type cells, even in the absence of extracellular 5-HT and regardless of 5-HT receptor activation [85]. Further experiments will be required to confirm this finding and identify the underlying mechanism. It is possible that the amount of monoamine transmitters packaged per individual vesicle is reduced in $SERT^{-/-}$ mice. However, the 80% reduction in 5-HT accounts for only a very small fraction (< 0.25%) of the total monoamines and the catecholamine content was unchanged in $SERT^{-/-}$ mice [85]. Alternatively, loss of SERT might favor partial rather than full release of the vesicular content. The properties and stability of the initial fusion pore that forms between the vesicle and extracellular space to allow efflux of transmitters can be regulated [117, 118]. This can result in transient fusion events (sometimes referred to as "kiss-and-run" exocytosis) that release only some of the vesicle cargo. In chromaffin cells the prevalence of these transient fusion events can be modulated by cellular firing patterns, G proteins, kinases, and remodeling of the actin cytoskeleton [100, 101, 117, 119-121]. Notably, different peptide cargo in the vesicles can also alter fusion kinetics and quantal size [122]. Perhaps the presence of intracellular and/or intravesicular 5-HT in wild-type cells somehow favors more complete emptying of vesicle contents. 5-HT has been reported to act as an intracellular messenger following SERT-mediated uptake in smooth muscle, platelets, and pancreatic β-cells [123-127]. This appears to involve transglutaminase-mediated covalent modification of proteins such as actin and monomeric G proteins (termed "serotonylation") [128-130]. In both platelets and β-cells one effect of serotonylation is to augment exocytosis, but whether this novel signaling paradigm

contributes to serotonergic control of catecholamine secretion or other chromaffin cell functions remains an open question.

Stress-evoked transcriptional regulation of tyrosine hydroxylase is altered in SERT knockout mice

Tyrosine hydroxylase is the rate limiting enzyme for catecholamine synthesis. Acute stress leads to increased expression / function of tyrosine hydroxylase in the adrenal medulla, and this is postulated to maintain catecholamine stores in the face of increased demand [40, 131-135]. Stress-evoked catecholamine secretion and upregulation of tyrosine hydroxylase both depend on the neuropeptide PACAP which is released from preganglionic splanchnic nerve terminals and acts on PAC1 receptors expressed on adrenal chromaffin cells [133, 136, 137]. Baseline (resting) plasma catecholamine levels were similar in wild type and SERT$^{-/-}$ mice [16]. Notably, catecholamine secretion evoked by restraint stress was enhanced in SERT$^{-/-}$ mice compared to wild-type controls, but SERT$^{-/-}$ mice failed to upregulate expression of tyrosine hydroxylase or angiotensin II receptors [17, 138]. Moreover, adrenal catecholamine content was reduced in the SERT$^{-/-}$ mice but not in wild type controls following restraint stress [16, 17]. Thus, two parallel effects of acute stress on chromaffin cells that are mediated by PACAP were differentially altered in the SERT$^{-/-}$ mice. The relationship between SERT and upregulation of tyrosine hydroxylase is intriguing and its nature remains to be elucidated. Stress also increases expression of several neuropeptides including galanin, NPY, and enkephalin; it will be interesting to determine if these changes are also disrupted by loss of SERT, and if local serotonergic signaling in the adrenal chromaffin cells is involved in this phenomenon. Clinically relevant doses of selective 5-HT reuptake inhibitors result in ≈80 - 90 % occupancy of SERT in the CNS [139, 140]. Assuming a similar occupancy in the adrenal gland, this could have a substantial impact on serotonergic regulation of adrenal chromaffin cells. Thus, determining if stress evoked transcriptional regulation is altered by SSRIs is also pertinent.

Is stress-evoked catecholamine secretion fine-tuned by serotonergic signaling/autocrine regulation?

There are several autocrine signals that can control adrenal chromaffin cells, but we postulate that the properties of 5-HT mediated inhibition are tuned to control stress-evoked secretion. One autocrine mediator is catestatin, a neuropeptide produced by proteolytic cleavage of chromogranins in large dense core secretory vesicles of chromaffin cells [141]. Catestatin is a noncompetitive antagonist of nicotinic ACh receptors, and inhibits secretion evoked by ACh, but has no effect on secretion evoked by other stimuli such as direct membrane depolarization or PACAP [141, 142]. Adrenal chromaffin cells also express GPCRs for a variety of autocrine, paracrine, endocrine, and neuronal transmitters. These include inhibitory autocrine receptors for catecholamines (α_2 adrenergic), ATP (P2Y purinergic) and opioids (μ-opioid), and receptors for paracrine signals including inflammatory mediators (e.g. prostaglandin EP3 receptors) [143-147]. These receptors are all thought to act predominantly by inhibition of I_{Ca} [72, 93]. In contrast, 5-HT$_{1A}$ receptors do not modulate Ca^{2+} channels, K^+ channels, or intracellular Ca^{2+} signaling in chromaffin cells [85]. We

postulate that the distinct mechanism of 5-HT_{1A} receptors, coupled with the modulatory influence of SERT, might fine-tune serotonergic signaling to control catecholamine secretion during periods of intense stimulation (i.e. stress) (Figure 3). With basal or modest stimulation the secretory vesicles release substantial amounts of ATP along with catecholamines, neuropeptides, and 5-HT. SERT-mediated clearance of the 5-HT would prevent activation of the 5-HT_{1A} receptors whereas P2Y purinergic receptors, activated by released ATP, robustly inhibit Ca^{2+} channels and thus exocytosis [143, 146, 148, 149] (Figure 3A). Endogenous opioids and catecholamines might also mediate autocrine inhibition of Ca^{2+} channels and exocytosis through activation of μ-opioid and α_2 adrenergic receptors respectively. During more intense / sustained stimulation (i.e. stress) several factors change that favor a dominant role for serotonergic modulation. GPCRs can recruit several pathways to inhibit Ca^{2+}channels, but the most widespread mechanism involves direct binding of Gβγ to the pore forming α_1 subunit of the channels [150, 151]. One notable feature of this "voltage-dependent inhibition" is that strong depolarization / repetitive firing can reverse Gβγ binding and thus channel inhibition [152-157] (for review see [91, 158]). Channel inactivation can also be slowed by Gβγ [159, 160]. Together, the altered inactivation, reversal of Gβγ binding, and build-up of residual Ca^{2+} within the cells are predicted to diminish the effectiveness of autocrine inhibition by P2Y receptors and other GPCRs that target Ca^{2+}channels during intense / sustained stimulation (Figure 3B). On the other hand, increased stimulation would lead to greater amounts of 5-HT being released that could overcome the opposing influence of SERT and activate the 5-HT_{1A} receptors. Since the 5-HT_{1A} receptors act through a distinct mechanism that does not involve Ca^{2+} channels or membrane excitability, the serotonergic inhibition might persist and become the dominant mechanism for feedback control during stress-like stimuli (Figure 3B). If plasma membrane SERT levels or transport activity are changed rapidly, or as a consequence of prolonged chromaffin cell activation, additional opportunities to modulate 5-HT_{1A} signaling could emerge.

Another consideration is that the neuropeptide PACAP might supplant acetylcholine (ACh) as the dominant excitatory transmitter at the splanchnic nerve – chromaffin cell synapse during stress-like stimuli [133, 136, 137]. Thus, any autocrine inhibition of chromaffin cells by catestatin will be lost because the peptide blocks nicotinic ACh receptors but has no effect on PACAP-evoked secretion [141, 142]. Furthermore, the stimulus-secretion coupling pathway recruited by PACAP is distinct from that of cholinergic stimulation and might not be effectively regulated by the autocrine GPCRs. Acetylcholine acts via nicotinic ACh receptors to depolarize the adrenal chromaffin cells, fire action potentials, and open the high voltage-activated Ca^{2+} channels that are modulated by GPCRs. Muscarinic receptors will also be activated and might contribute to stimulus-secretion coupling [161]. In contrast, PACAP acts on the PAC1 GPCR which has complex downstream signaling through both Gs and Gq-type G protein pathways [162]. In chromaffin cells PACAP is a potent secretagogue; it causes modest membrane depolarization and sustained Ca^{2+}entry through poorly defined channels that might include low-voltage-activated $Ca_V3.2$ (T-type) channels, TRP channels, and perhaps other pathways [163-166]. Thus, the Ca^{2+}entry pathways recruited by PACAP are distinct from those activated by cholinergic stimulation and might not be effectively regulated by the autocrine GPCRs that target Ca_V2 voltage-gated Ca^{2+} channels. In contrast,

the 5-HT_{1A} receptors might still mediate autocrine inhibition of PACAP-evoked secretion (Figure 3B).

Further experiments are needed to directly test the above model for autocrine regulation of adrenal chromaffin cells by 5-HT, to dissect the molecular mechanism(s) utilized by 5-HT_{1A} receptors, and to determine if serotonergic signaling inhibits PACAP-evoked catecholamine secretion. However, it is clear that the components for autocrine regulation of adrenal chromaffin cells by 5-HT are in place and that SERT plays an important role in this pathway. First, SERT is responsible for accumulating 5-HT into the chromaffin cells; second, the transporter constrains the ability of 5-HT to recruit the 5-HT_{1A} receptor-mediated inhibition [85]. Consequently, the loss of SERT function might have complex effects. Acute block of SERT is predicted to enhance serotonergic inhibition by removing the constraint for receptor activation. The effects of chronic loss or block of SERT might be even more complex, and derive in part from a loss of the local source of 5-HT that drives autocrine feedback. As demonstrated in the $SERT^{-/-}$ mice, chronic loss of SERT depletes 5-HT from chromaffin cells [16, 82, 85] , which we suggest will diminish or preclude autocrine serotonergic inhibition. Indeed, $SERT^{-/-}$ mice display an exaggerated increase in plasma epinephrine evoked by acute restraint stress [16, 17], and chronic block of SERT with SSRIs enhances the increase in plasma epinephrine produced by hypoglycemia in rats and humans [13-15]. These phenotypes are consistent with loss of autocrine inhibition within the adrenal gland, although the global nature of the knockout / block of SERT means that other sites could also contribute; loss of SERT in the CNS could alter central drive to the peripheral sympathetic nervous system, and 5-HT will also be depleted from other tissues including platelets.

Future direction exploiting novel transgenic mouse models

The $SERT^{-/-}$ mouse is a valuable model for investigating serotonergic signaling and the mechanisms of antidepressants and other drugs that target SERT [3, 18, 167, 168]. However, the global nature of the $SERT^{-/-}$ knockout makes it difficult to determine the site(s) where SERT might be acting to control the sympathetic stress response. As discussed in this article, SERT could act at multiple sites within the brain and spinal cord to control central drive to the peripheral sympathetic nervous system. SERT is also expressed in the adrenal chromaffin cells, and a subset (~30%) of sympathetic neurons in the rat superior cervical ganglion is reported to express SERT [20]. It will be important to establish if the expression and function of SERT is conserved in human adrenal chromaffin cells. Mechanistically, it remains unclear which site(s) of SERT expression are most pertinent for controlling the sympathetic stress response. Recent advances that enable conditional, tissue-selective excision of SERT using Cre/LoxP technology promise to provide new insight into this question [169]. In the initial report using this approach, SERT expression was efficiently reduced in CNS serotonergic neurons by crossing *ePet-Cre* mice with the "*floxed*" SERT mice [169]. Notably, Cre is not expressed in the adrenal gland of *ePet-Cre* mice [170], so SERT will remain intact in the peripheral sympathoadrenal system in this model. We have performed preliminary experiments with the goal of generating the complementary model, where SERT is selectively excised in the peripheral sympathetic nervous system, but not the CNS. As SERT is not expressed in catecholaminergic neurons in the CNS, we initially crossed floxed SERT

mice with *TH-Cre* mice (mice expressing Cre driven by the tyrosine hydroxylase promoter) [171]. As expected, Cre is robustly expressed in adrenal chromaffin cells and catecholaminergic cells in the peripheral and central nervous systems. It is also expressed in some other CNS neurons, presumably due to transient expression of TH during development [171]. Nonetheless, our preliminary data show that the SERT gene is excised from adrenal chromaffin cells but remains intact in the CNS (unpublished observation) (Figure 4). One could also imagine the use of temporally-restricted transgenic or viral approaches to limit SERT excision in the peripheral nervous system to adult animals, lessening concerns of developmental effects. Together, these approaches have the capacity to reveal pertinent and functionally distinct sites of SERT action and directly test the hypothesis that adrenal chromaffin cells form a peripheral hub for serotonergic regulation of the sympathoadrenal stress response.

It is well-documented that SERT function and its expression at the plasma membrane can be regulated by genetic polymorphisms, promoter variants, and cell signaling pathways [1, 2, 18, 172, 173]. Little is known about what impact this might have on sympathoadrenal chromaffin cells, although the response to acute restraint stress was similar in heterozygous $SERT^{+/-}$ and wild type mice whereas this response was enhanced in $SERT^{-/-}$ mice [16]. As noted above, interpretation of this observation is complicated because of the global nature of the genetic deletion in this model and conditional heterozygous mice might provide additional insight. Other transgenic mice have been engineered to model human variants with altered SERT function [1]. One such model bears a single, autism-associated missense mutation in SERT (Gly56Ala) that confers elevated transport function. These mice display hyperserotonemia, deficits in social interactions, repetitive behavior, and altered gastrointestinal function [174, 175]. Determining how these and other (patho)physiological alterations of SERT impact the sympathoadrenal stress response both *in vivo* and at a cellular / mechanistic level could provide important insights into the autonomic dysfunction associated with depression, anxiety, and other disorders in which serotonergic signaling is disrupted.

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Abbreviations

5-HT	5-hydroxytryptamine
ACh	acetylcholine
Epi	epinephrine
NE	norepinephrine
GPCR	G protein coupled receptor
SERT	serotonin transporter

SERT$^{-/-}$	SERT knockout mice
SSRI	selective serotonin reuptake inhibitor

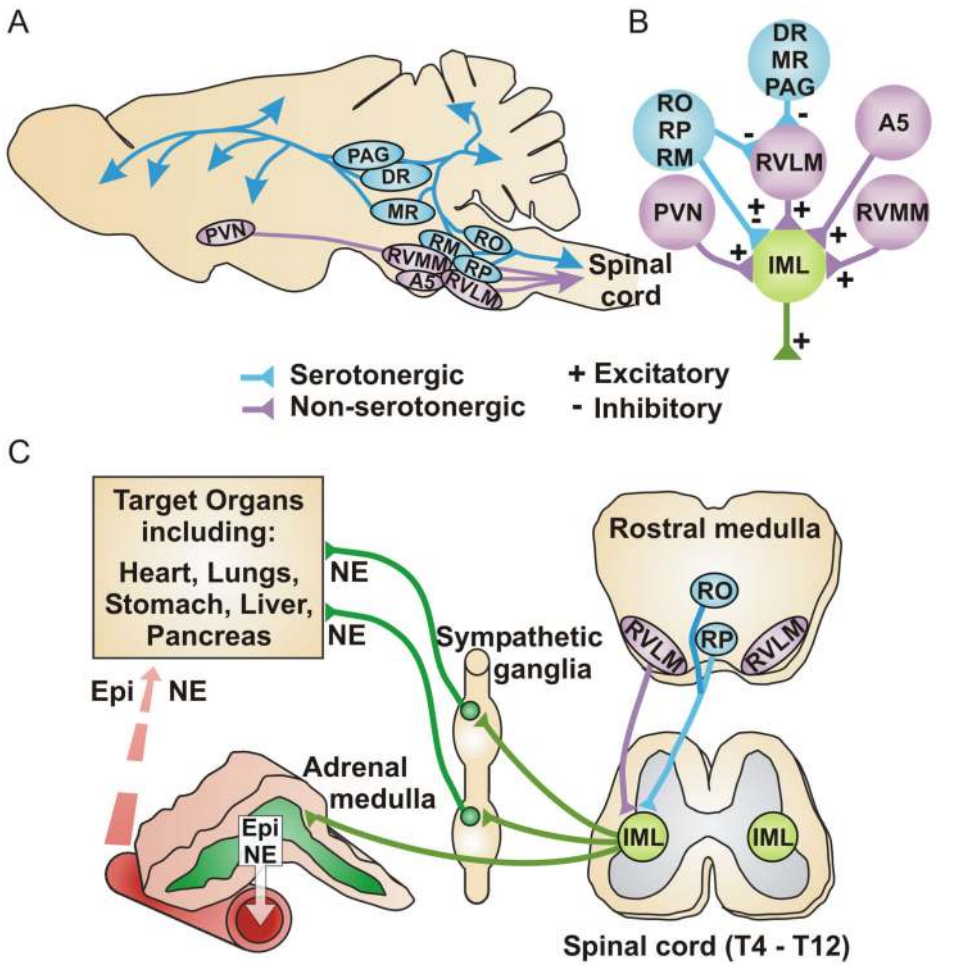

Figure 1. Relationship between central serotonergic neurons and the sympathetic nervous system

A) Simplified schematic of an adult rodent brain showing serotonergic nuclei (blue) and other brainstem regions (purple) important for central drive to the peripheral sympathetic nervous system. Abbreviations: PAG periaqueductal grey; DR dorsal raphe; MR median raphe; RO raphe obscurus; RM raphe magnus; RP raphe pallidus; PVN paraventricular nucleus of the hypothalamus; RVMM rostral ventromedial medulla; RVLM rostral ventrolateral medulla; A5 A5 noradrenergic cell group. **B)** Simplified schematic showing that preganglionic sympathetic neurons in the intermediolateral column (IML) of the thoracic spinal cord receive central drive from the RVLM and other brainstem / forebrain regions. The RVLM integrates input from multiple regions but only serotonergic input is shown; this is predominantly inhibitory, although some excitation might also occur. Serotonergic nuclei also send projections directly to the spinal cord to modulate central drive. **C)** Simplified schematic showing descending projections from the rostral medulla to the preganglionic sympathetic neurons in the intermediolateral column of the thoracic spinal cord (IML). Preganglionic neurons in the IML project to the peripheral sympathetic ganglia and the adrenal medulla. Postganglionic sympathetic neurons project from the ganglia to specific target organs and release norepinephrine (NE) to exert local control. Adrenal chromaffin cells comprise the neuroendocrine arm of the sympathetic nervous system and

exert widespread effects by secreting epinephrine (Epi), norepinephrine (NE), and various neuropeptides into the bloodstream.

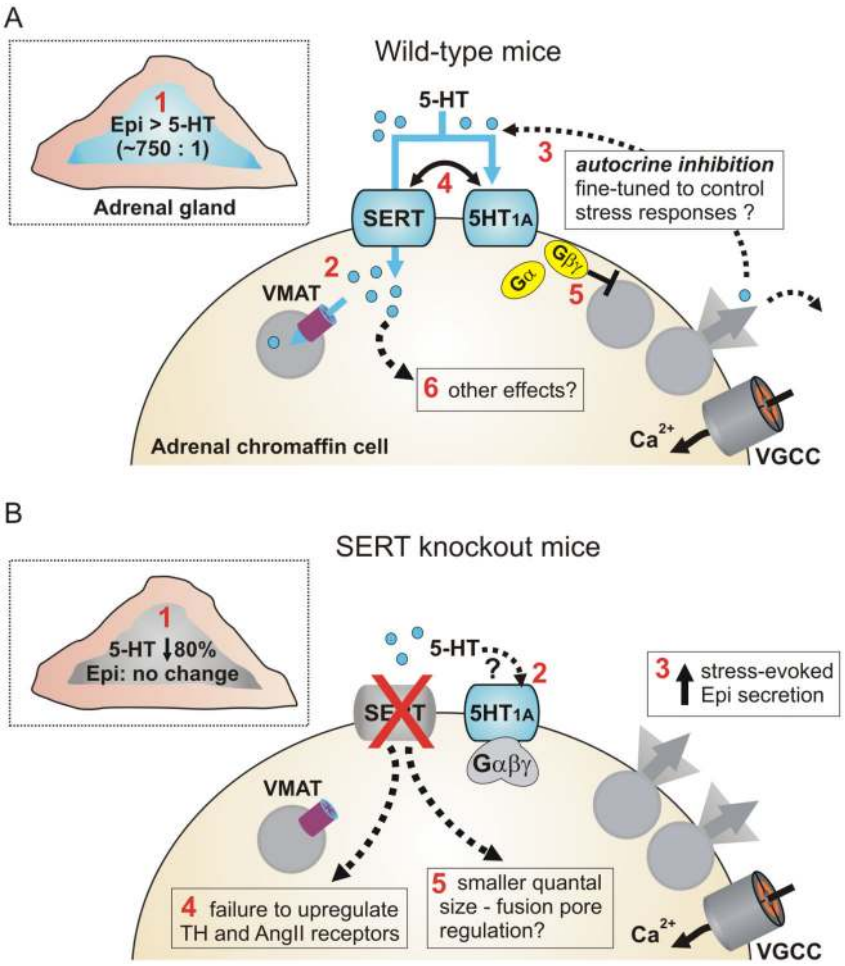

Figure 2. SERT and serotonergic signaling in adrenal chromaffin cells

A) Schematic showing the mouse adrenal gland (inset) and an adrenal chromaffin cell. SERT can exert multiple effects indicated by the red numbering. 1) SERT is expressed in the adrenal medulla (blue) but not the adrenal cortex. 5-HT is found in the adrenal gland largely due to SERT-mediated uptake. Levels of 5-HT are approximately 1/750th of epinephrine levels. 2) Following SERT-mediated uptake into chromaffin cells 5-HT is packaged into secretory vesicles by the vesicular monoamine transporter (VMAT). 3) Chromaffin cells also express 5-HT$_{1A}$ receptors providing for potential autocrine regulation by 5-HT. 4) SERT constrains the ability of 5-HT to recruit the 5-HT$_{1A}$ receptor signaling pathway. 5) The 5-HT$_{1A}$ receptors reduce catecholamine secretion but this does not involve the typical mechanisms used by inhibitory GPCRs that target chromaffin cell voltage-gated Ca^{2+} channels (VGCC), K^+ channels, or Ca^{2+} signaling. 6) Other effects of SERT and/or intracellular 5-HT are also possible. **B)** Known and potential effects of SERT knockout on adrenal chromaffin cells. 1) The adrenal catecholamine content is unaltered in glands from SERT$^{-/-}$ mice, but the 5-HT content is reduced by ≈80%. 2) 5-HT$_{1A}$ receptors are still present and functional, but the loss of cellular 5-HT content presumably prevents autocrine inhibition via these receptors. Conversely, other sources of 5-HT might be expected to more efficiently recruit the 5HT$_{1A}$ receptors as the opposing action of SERT is no longer present.

3) The sympathoadrenal response to acute restraint stress (increase in plasma epinephrine) is exaggerated in SERT knockout mice. 4) There is a failure to upregulate expression of tyrosine hydroxylase (TH) or angiotensin II (Ang II) receptors in response to acute stress in SERT knockouts. 5) The quantal size of unitary vesicular fusion events detected using carbon fiber amperometry is reduced in cells isolated from $SERT^{-/-}$ mice.

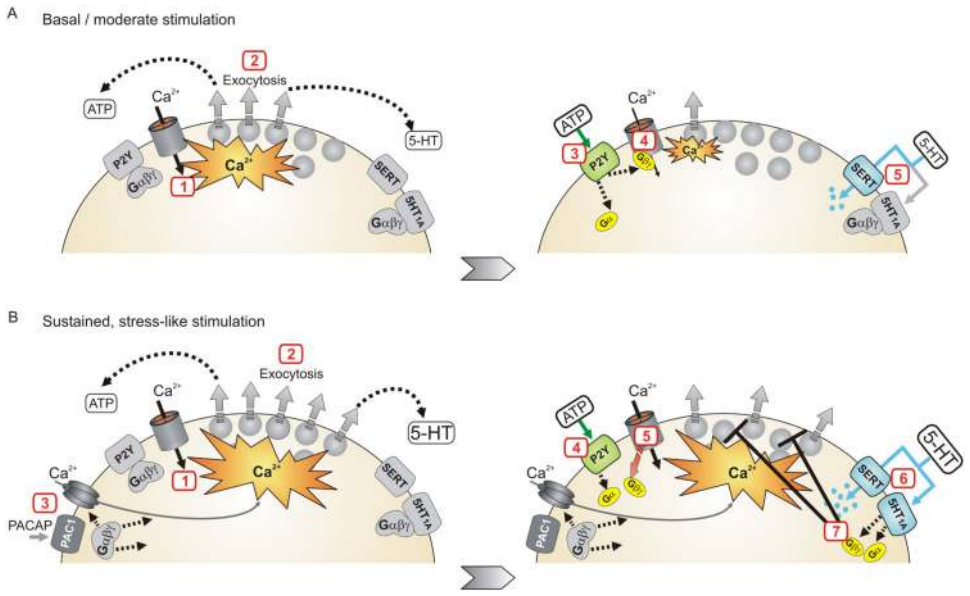

Figure 3. Proposed model in which serotonergic inhibition is fine-tuned to control stress-evoked catecholamine secretion

A) During periods of basal or brief stimulation purinergic (and other) receptors that inhibit voltage-gated Ca^{2+}channels mediate autocrine regulation of exocytosis. *Left panel*: 1) Acetylcholine released from the splanchnic nerve depolarizes chromaffin cells opening voltage-gated Ca^{2+} channels. 2) The Ca^{2+} entry triggers exocytosis of catecholamines and other transmitters including ATP, opioids, and 5-HT. *Right panel*: 3) ATP activates P2Y autoreceptors. 4) Gβγ inhibits the Ca^{2+} channels, reducing Ca^{2+} entry and thereby exocytosis. 5) SERT mediated uptake of 5-HT opposes activation of the 5-HT_{1A} receptors so this signaling pathway is not recruited. **B)** With sustained, stress-like stimulation 5-HT signaling becomes the dominant pathway for autocrine regulation. *Left panel*: 1-2) As in panel A, acetylcholine released from the splanchnic nerve depolarizes chromaffin cells opening voltage-gated Ca^{2+} channels and triggering exocytosis. 3) With sustained stimulation PACAP might also be released from the splanchnic nerve and become the main stress mediator activating the chromaffin cells. PACAP acts via PAC1 receptors which recruit additional Ca^{2+} entry pathways perhaps including Ca_V3 (T-type) channels and TRPC channels. *Right panel*: 4) ATP activates P2Y autoreceptors. 5) Inhibition of Ca^{2+} channels becomes ineffective with strong, sustained depolarization in part because Gβγ dissociates from the channels. 6) The stronger stimulation leads to greater increase in local 5-HT and activation of 5-HT_{1A} receptors. 7) The 5-HT_{1A} receptors inhibit exocytosis by an atypical mechanism independent from cellular excitability / Ca^{2+} entry that can persist during the sustained stimulation. In this manner, 5-HT signaling becomes the dominant pathway for autocrine regulation.

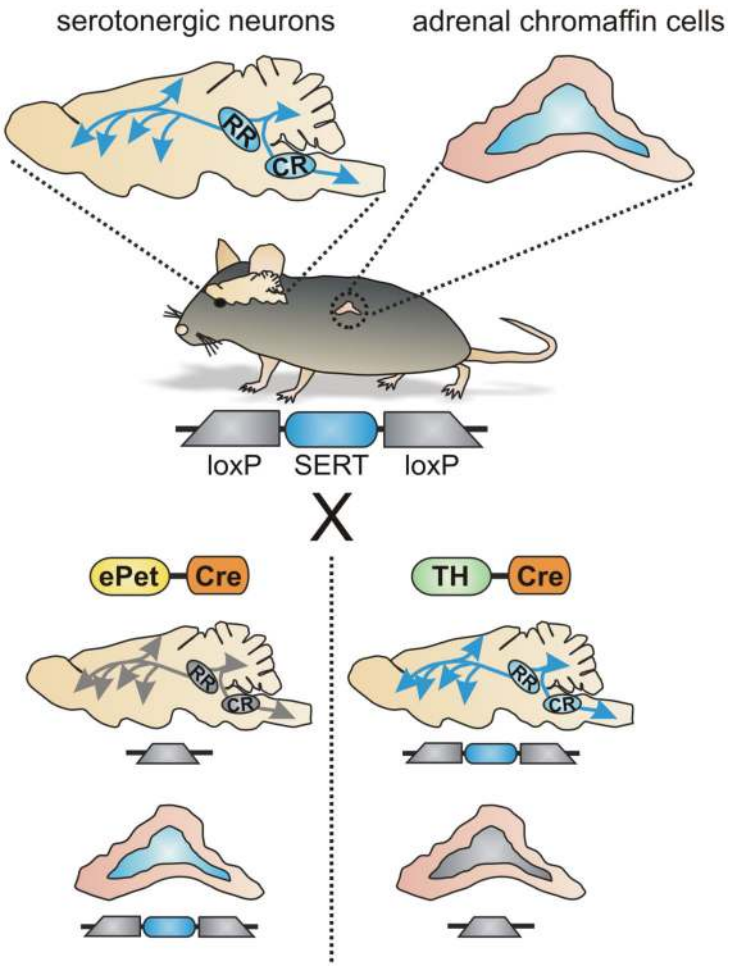

Figure 4. Schematic depicting use of transgenic mice to selectively excise SERT from the peripheral sympathoadrenal system or central serotonergic neurons

In the "floxed" SERT mouse, SERT is expressed in both central serotonergic neurons and adrenal chromaffin cells (depicted by blue shading). Crossing these mice with Cre driver lines provides a strategy to selectively excise SERT in a tissue specific manner. For example, *ePet-Cre* drives expression of Cre and excision of SERT in serotonergic neurons of the CNS but not in adrenal chromaffin cells. Conversely, using *TH-Cre* will result in excision of SERT in the adrenal chromaffin cells but not in CNS raphe neurons.