● **PERSPECTIVE**

Metabolic recruitment of spinal locomotion: intracellular neuromodulation by trace amines and their receptors

The trace amines (TAs) are a family of endogenous amines with structural, metabolic, physiological and pharmacological similarities to classical monoamine neurotransmitters. The TA family includes tyramine, octopamine, β-phenylethylamine (PEA), and tryptamine (**Figure 1**). Their presence has been well documented in all vertebrate and invertebrate species studied. Like the classical monoamines, the TAs are synthesized *via* enzymatic decarboxylation of the precursor aromatic amino acids phenylalanine, tyrosine, and tryptophan with aromatic L-amino acid decarboxylase (AADC; also called DOPA decarboxylase). For decades, researchers have known that these amines are present in the brain. A large earlier literature asserted their role as endogenous neuromodulators of monoaminergic excitability and neurotransmission, challenging the more conventional view of the TAs as metabolic byproducts that interfere with classical monoamine modulatory functions (Berry, 2004).

TA rates of synthesis are equivalent to that of dopamine and nor-adrenaline. However, unlike the classical monoamines, the TAs have an exceedingly rapid turnover rate, seen as an endogenous pool half-life of approximately 30 seconds. This is because in the central nervous system (CNS), the TAs are not stored as a reserve pool in vesicles, so measured total content is in much lower 'trace' quantities. Still, the TAs circulate in cerebrospinal fluid at levels similar to the classical monoamines and have a heterogeneous distribution (Berry, 2004). They are metabolized *via* monoamine oxidases (MAOs) and MAO inhibitors lead to rapid and significant TA accumulations indicative of their high synthesis rate, and demonstrating TAs as physiologically regulated.

The discovery in 2001 of G-protein coupled trace amine-associated receptors (TAARs) preferentially activated by TAs established mechanisms by which TAs can produce effects of their own, with tyramine and PEA activating TAAR1, and PEA and tryptamine activating TAAR4 (Borowsky et al., 2001). A clear role for TA actions on CNS TAAR1 receptors was supported by more recent observations using selective TAAR1 pharmacology and TAAR1 knockout mice. Overall, TAAR1 activity appears to depress monoamine transport and limit dopaminergic and serotonergic neuronal firing rates *via* interactions with their presynaptic autoreceptors (Leo et al., 2014).

Is there a specific spinal cord TA-ergic neuronal circuit linked to locomotion? The discovery of TAARs expressed in the CNS also introduced the possibility of uncharacterized CNS TA-ergic neuronal systems. Candidate TA-ergic neurons include 16 anatomically segregated collections of D cells that contain the essential TA synthesis enzyme (AADC) but no other monoamine synthesis enzymes. The largest cluster of D cells, called D1 cells, were found in the spinal cord distributed along the central canal, primarily in lamina X (Jaeger et al., 1983). Ultrastructural identification of synapses and secretory vesicles confirmed D1 cells as neurons. At least one of their processes projects into the lumen of the central canal, which makes them part of a group of cerebrospinal fluid-contacting neurons. D1 cells may function to monitor cerebrospinal fluid (CSF) related events and relay the

information into modulatory commands for the motor system. After spinal cord injury (SCI), AADC-expressing D cells facilitate spinal motor excitability by increasing their expression of monoamines (Wienecke et al., 2014). Notably, a morphologically similar population of neurons activates locomotor circuits in larval zebrafish (Wyart et al., 2009).

We explored a role for the TAs in the neuromodulation of rat spinal cord locomotor generating circuits (Gozal et al., 2014). We showed that the spinal cord contains the substrates for TA biosynthesis (AADC) and for receptor-mediated actions *via* trace amine-associated receptors (TAARs) 1 and 4. We next examined TA actions on motor activity using the *in vitro* isolated neonatal rat spinal cord. Tyramine and tryptamine most consistently increased motor activity with prominent direct actions on motoneurons. In the presence of N-methyl-D-aspartate, all applied TAs supported expression of a locomotor rhythm that was indistinguishable from that ordinarily observed with serotonin (5-HT). This suggested actions on common central pattern generating neurons (**Figure 2A**). The TAs also generated distinctive complex rhythms characterized by episodic bouts of locomotor-like activity that supported recruitment of additional circuits (**Figure 2B**). TA actions on locomotor circuits did not require interaction with descending monoaminergic projections since evoked motor rhythms were maintained following block of all Na$^+$-dependent monoamine transporters or the vesicular monoamine transporter. Instead, TA (tryptamine and tyramine) actions depended on intracellular uptake *via* pentamidine-sensitive Na$^+$-independent membrane transporters. Requirement for intracellular transport was consistent with the TAs having much slower locomotor onset than 5-HT and for activation of intracellular TAARs. Behaviorally, the actions of applied TAs integrated well with their known pharmacological sympathomimetic function. To test for endogenous actions following biosynthesis, we increased intracellular amino acid levels with cycloheximide. Locomotor-like activity emerged and included distinctive TA-like episodic bouts. Putative cellular transport mechanisms are outlined in **Figure 2C**. Overall, both our anatomical and functional evidence supported a role of the TAs as an intrinsic spinal monoaminergic modulatory system that was capable of promoting recruitment of locomotor circuits independent of the descending monoamines. Provided evidence of a spinal cord substrate for TAs with independent intrinsic biological actions supported the TAs as *bona fide* endogenous monoamines neuromodulators with their own unique neuromodulatory status.

Possible functional roles of trace amine signaling: Intracellular transport appears to be a prominent requirement for observed TA actions, and this suggests that intracellularly synthesized TAs may act to intrinsically modulate their own function independent of external neuronal interactions. This occurs presumably *via* TAAR-mediated changes in signal transduction pathways that modulate cellular/synaptic activity (**Figure 2C**). If intracellular TA biosynthesis was regulated by subcellular substrate precursor amino acid availability for subsequent intracellular TAAR activation, increases in activity may follow activation of amino acid mobilizing catabolic pathways. In this way intracellularly-synthesized TAs may comprise an integral cellular metabolic cascade for temporary augmentation of motor activity. For example, such a cascade could be a component part of an innate multi-organ system autonomic sympathetic stress response (*e.g.*, fight or flight). This is consistent with TAARs being important olfactory odorant receptors in innate survival responses (Liberles, 2015). It is also consistent with observed sympathomimetic cardiovascular actions (Broadley, 2010). In
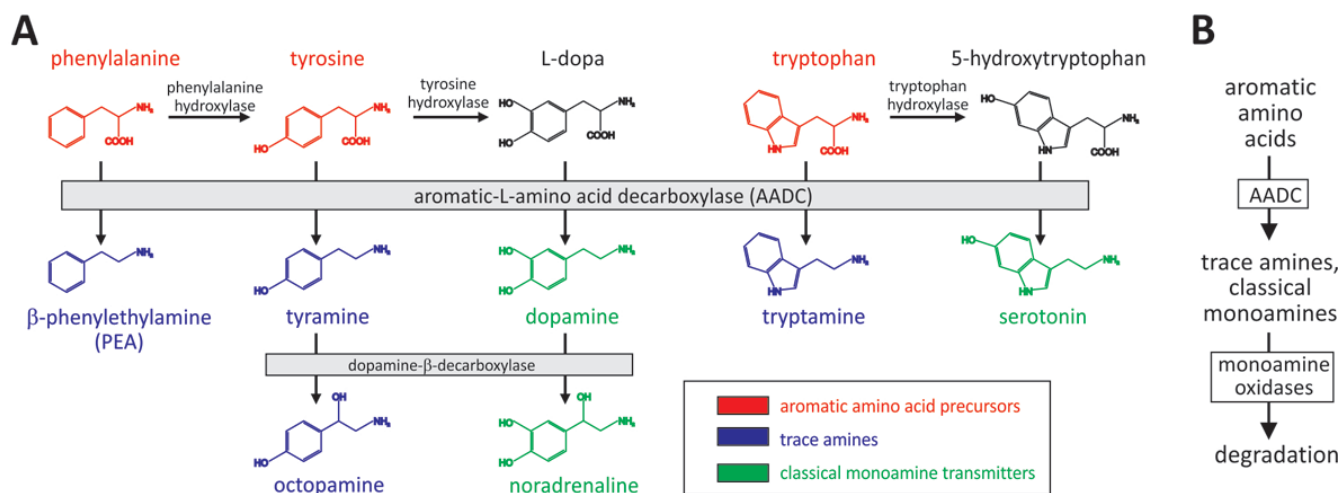
Figure 1 Comparison of monoamine synthesis pathways.
(A) The trace amines (TAs) are a group of endogenous monoamines that include tryptamine, tyramine, octopamine and β-phenylethylamine (PEA; blue). The TAs have structural, metabolic, physiologic, and pharmacologic similarities to the classical monoamine transmitters (green) and are synthesized from the same precursor aromatic amino acids (red). Unlike the classical monoamines, aromatic-L-amino acid decarboxylase (AADC; also called dopa decarboxylase) is the only enzyme required to produce them. Conversion from the TAs to the monoamines does not appear to occur. (B) Overview showing that, like the classical monoamine transmitters, the TAs are degraded by the monoamine oxidases.

this context, intrinsic TA facilitation of the locomotor system may arise from phylogenetically primitive innate locomotor escape responses that reside completely within the spinal cord. Metabolic mechanisms that promote response amplification would clearly be adaptive in survival responses.

The previously described high TA synthesis rates may provide an additional important clue to their function. High synthesis rates support rapidly adjustable shifts in TA levels that are exquisitely attuned to moment-to-moment fluctuations in bioavailable precursor amino acid substrate availability. Alternatively, assuming full substrate availability, signal transduction-mediated alterations in AADC activity could serve to adjust endogenous TA levels and act as a variable gain modulator of circuit function. This would apply anywhere there was TAAR1 and /or TAAR4 expression in AADC$^+$ neurons. Moreover, if the TAs are bi-directionally transported across membranes by widely expressed transporters (**Figure 2C**), neurons expressing TAAR1 and/or TAAR4 could be modulated by nearby AADC$^+$ TA-producing cells. As AADC is expressed in spinal cord vasculature (Gozal et al., 2014; Li et al., 2014) and expression in microvasculature is strongly modifiable (Li et al., 2014), the circulatory system may afford diffuse TA delivery for broad TA-based modulation.

Proposed future studies: Future studies are required to validate and extend our initial findings and would be aided with transgenic approaches and the use of selective TAAR agonists and antagonists. One immediate question is whether the TAs integrate and interact with conventional monoamines to modulate the locomotor system. The answer is yes, as we have observed that TA actions are broadly facilitatory to ongoing 5-HT-induced locomotion (Gozal and Hochman; unpublished observations). Experimental support for modulatory actions of the TAs on motor behavior in adult preparations would importantly extend our observations in neonates. Perhaps most essential is to undertake experiments that mechanistically-link the chain of hypothesized endogenous events. This includes; (i) identification of the physiological trigger that increases bioavailable aromatic amino acids, (ii) subsequently increasing endogenous TA synthesis levels, (iii)

activating TAARs, and (iv) facilitating locomotor activity. A conceptual framework related to mechanisms of cellular stress may be a good starting point for such studies.

Possible neurotherapeutic interventions: As the TA/TAAR modulatory system may help set the baseline excitability of spinal motor systems, therapeutic elevations or reductions in TAAR signaling may help control aberrant motor excitability in various disease states. For example, it may turn out that the TA/TAAR modulatory system is better at temporal motor gain control than the classical monoamines.

Unfortunately, while mechanisms that selectively alter endogenous TA levels may have neurotherapeutic relevance, their synthesis and degradative enzymes are common to the classical monoamines. Thus, elevating expression levels *via* block of degradation (*e.g.*, MAO inhibitors) would also alter classical monoamine content and actions. However, selective signal transduction-mediated alterations in AADC activity could selective have more preferential impact on the TAs (Berry, 2004). Alternately, if substrate availability is rate-limiting for TA biosynthesis (*e.g.*, but not for the classical monoamines with vesicle pool reserves), simply increasing substrate availability with dietary precursor amino acid supplementation, or providing TAs directly (*e.g.*, elevated in chocolate, aged cheeses, and wines) could have functional consequences on motor circuits of neurotherapeutic relevance (Jackson, 1975).

Classical monoamine receptor agonists improve locomotor functional outcome after SCI in animal models (Courtine et al., 2009). We demonstrate that the TAs act as an intrinsic spinal cord monoaminergic modulatory system. They recruit locomotor patterns that include unique episodic events not activated by the classical monoamines (Gozal et al., 2014). This, and our unpublished observations that they facilitate ongoing 5-HT-induced locomotion, supports consideration of TAs or TAAR receptor activation in the management of SCI with compromised descending monoaminergic systems. For example, strategically-timed delivery of aromatic amino acid precursors and/or TA dietary supplements for SCI patients could improve motor performance, including motor endurance by their cardiovascular
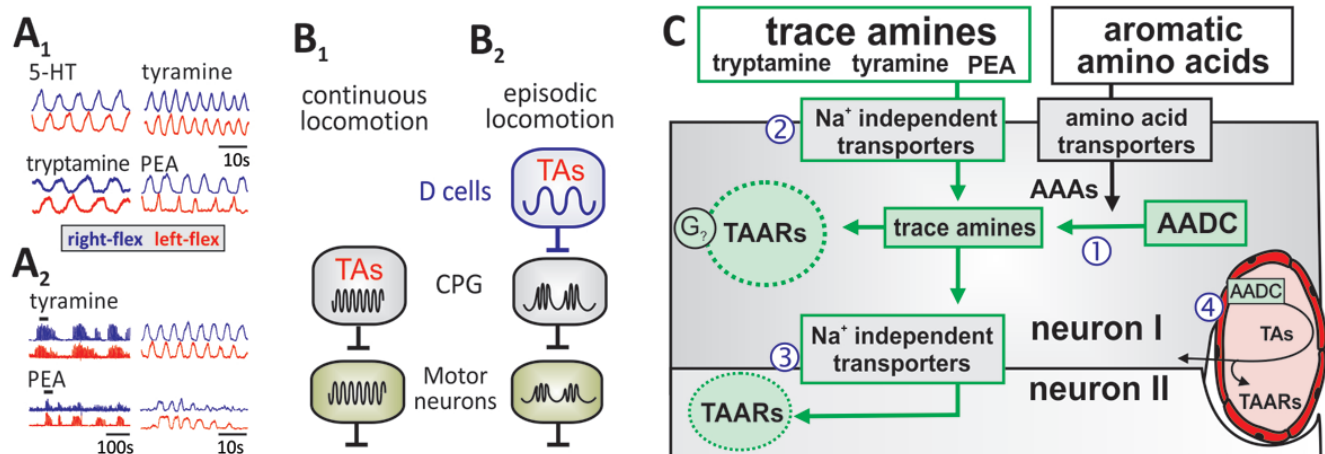
Figure 2 Example activity patterns (A), hypothetical network (B) and cellular pathways (C) for trace amine-mediated actions.
(A$_1$) Examples of continuous locomotor rhythms generated in the presence of 5-hydroxytryptamine (5-HT) and the trace amines (TAs). Shown are smoothed activity pattern envelopes reporting activity for right (blue) and left flexors (red). (A$_2$) Episodic bouts of locomotor-like rhythms are shown for tyramine and β-phenylethylamine (PEA) at slow (left) and expanded time scales (right). Bar over epochs at left identified expanded waveform at right. (B) Proposed circuit locations for TA-induced modulatory actions in the emergence of continuous and episodic locomotor rhythms. (B$_1$) TA modulatory actions intrinsic to locomotor central pattern generating neurons (CPG) would produce actions comparable to those seen with the descending neuromodulatory transmitter 5-HT. (B$_2$) TA-induced slower activity rhythms onto neurons that drive the CPG could lead to the episodic waxing and waning of rhythmic output to motor neurons. Candidate neurons are the lamina X AADC$^+$ D cells. (C) Putative transport and intracellular signaling mechanisms for observed TA actions. The TAs are synthesized from their precursor aromatic amino acids (AAAs) *via* the essential synthesis enzyme AADC ① . Intracellular TAs act on TAARs to produce G protein-coupled neuromodulatory responses. TA transport into neurons *via* Na$^+$-independent membrane transporters allows for trace amine-associated receptors (TAAR)-containing neurons to also be modulated by TA following their exogenous application, ② , (ii) release from AADC expressing neurons (③ ; here neuron I onto neuron II) or (iii) release from AADC-expressing endothelia ④ .

sympathomimetic actions (Broadley, 2010). Moreover, as the TAs have been shown to depress reflexes (Bowman et al., 1964), they may concomitantly reduce SCI-induced hyperreflexia and/or nociception.

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