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De novo biosynthesis of morphine in animal cells: An evidence-based model

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Summary

Recent empirical findings have contributed valuable mechanistic information in support of a regulated *de novo* biosynthetic pathway for chemically authentic morphine in animal cells, with many similarities to the extensively characterized multi-enzyme plant pathway in opium poppy (*Papaver somniferum*). The present review elaborates an evidence-based model of cellular morphine expression that reflects a coalescence of these recent biochemical data with historical data gleaned from over thirty years of neurochemical/neuropharmacological investigation into the etiology and biological significance of dopamine (DA)-related heterocyclic conjugate molecules, termed tetrahydroisoquinoline (TIQ) or benzylisoquinoline (BIQ) alkaloids, and with outstanding work completed over the last decade that has elucidated biochemical and molecular bases of morphine and related isoquinoline alkaloid expression in plant systems. In essence, we are now afforded a rare window of opportunity to firmly establish essential biochemical linkages between plant and animal biosynthetic pathways that have been conserved throughout evolution.

key words: **dopamine • tetrahydroisoquinoline • benzylisoquinoline • L-tyrosine • tyramine • tetrahydropapaveroline • nitric oxide • tyrosine hydroxylase • CYP2D6 • morphine**

Abbreviations: **DA** – dopamine; **TIQ** – tetrahydroisoquinoline; **BIQ** – benzylisoquinoline; **L-TYR** – L-tyrosine; **L-DOPA** – L-3,4-dihydroxyphenylalanine; **TA** – tyramine; **TDC** – L-TYR decarboxylase; **DDC** – L-DOPA decarboxylase; **THP** – tetrahydropapaveroline; **NO** – nitric oxide; **TH** – tyrosine hydroxylase; **CYP** – cytochrome P450

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BACKGROUND

The historical weight of morphine's dualistic potential to provide highly efficacious pain control, inextricably linked to debilitating side-effects and addiction, has maintained its ominous pride of place as a DEA Schedule II drug [1–5]. Curiously, initial speculations as to the existence and potential physiological role of endogenous morphine were made over 30 years ago by prominent researchers in the field of alcohol abuse, not opiate abuse, who advanced the hypothesis that the reinforcing or addictive effects of ethanol were functionally linked to the cellular effects of DA-derived isoquinoline alkaloids, notably the tetrahydroisoquinoline (TIQ) salsolinol [6–8] and the benzylisoquinoline (BIQ) morphine precursor (tetrahydropapaveroline) THP [9–11]. Recognition of TIQs, THP, and endogenous morphine as active principles of alcohol abuse was inherently linked to their normal presence in dopamine (DA)-ergic neurons, enhanced cellular expression following chronic ethanol intake [11–16], and concentration-dependent disregulation of DA metabolism and/or DA-ergic signaling in mesocortical/mesolimibic areas such as the nucleus accumbens (NAC) and ventral tegmental area (VTA) traditionally associated with reinforcement of alcohol-related behaviors [17–21]. The causal relationship and functional association of CNS expression of TIQ and BIQ alkaloids to alcohol abuse remains controversial despite anatomical, physiological, pharmacological, and behavioral evidence linking DA-ergic and opioidergic systems in limbic areas associated with reinforcement of ethanol intake behaviors [22–25].

The functional association between aberrant DA metabolism, cellular expression of isoquinoline alkaloids, and the etiology of Parkinson's Disease has also been extensively studied and debated for three decades [26–34]. In contrast to the hypothesized role of isoquinoline alkaloids to activate neural circuits involved in the reinforcement of alcohol dependence, these same conjugate molecules were proposed as pathophysiological agents responsible for Parkinson's Disease-associated symptomatology. Interestingly, by the early 1970s a functional association between L-3,4-dihydroxyphenylalanine (L-DOPA) therapy and *in vivo* formation of BIQs had been proposed [35–38]. It was subsequently demonstrated that urinary levels of morphine, codeine, and THP in L-DOPA-treated Parkinsonian patients are dramatically elevated as compared to matched controls and abstinent alcoholics [39]. Not surprisingly, enhanced production of THP in Parkinsonian patients was peremptorily linked to the mediation of adverse side effects and cellular toxicity evolving from chronic L–DOPA therapy [40–45], despite clinical evidence supporting positive effects of morphine on L-DOPA-associated dyskinesias [5,46–48].

By the mid 1970s, evidence of endogenous morphine expression in animal systems was provided by Spector and coworkers who characterized a nonpeptide morphine-like compound (MLC) extracted from mammalian brain which bound with high affinity and selectivity to an anti-morphine serum, originally intended for radioimmunoassay of morphine in blood and urine, and exhibited opiate-like inhibitory effects in established bioassays [49,50]. The same anti-morphine serum was employed to provide immunohistochemical detection of MLC in CNS areas including vestibular, cerebellar, and raphe systems [49]. Subsequently, Bianchi and coworkers replicated and extended the original anatomical observations of Spector and coworkers by demonstrating uptake and accumulation of 3H-labeled morphine within defined rat brain areas [51,52] and providing immunohistochemical localization of morphine-like material in perikarya, fibers, and terminals of neurons in discrete areas of both rat and human brain [51–53]. In the same study, morphine-like immunoreactive material associated with striatal neurons was markedly reduced following exposure of brain slices to high K+ concentrations [51,52], a physiologically important observation that was subsequently addressed in great depth in later studies from this same group [54,55].

FORMULATION OF AN EVIDENCE-BASED MODEL OF EUKARYOTIC MORPHINE BIOSYNTHESIS

1. Critical re-evaluation of historical data

Historically, anatomical observations of intrinsically low basal levels of immunoreactive morphine-like material widely distributed across diverse CNS areas may have led members of the scientific community to cursorily disregard any compelling argument in support of a biological role for endogenous morphine expression. Because CNS distributions of immunoreactive morphine-like material did not appear to be strictly co-localized with DA-ergic systems, there was also an apparent conflict with accumulated data linking increased or aberrant production of DA metabolites to randomly formed DA-derived BIQ alkaloids. Extensive data sets evolving from alcohol and Parkinson's Disease research introduced inconclusive, often contradictory, evidence indicating that non-physiological concentrations of isoquinoline alkaloids, often in the millimolar range, were required to mediate cellular toxicity via down-regulation of necessary DA metabolism and turnover linked to free radical production. Because biologically meaningful concentration of BIQ alkaloids were often observed to have little or no effect on DA metabolism and cellular integrity, a null hypothesis was apparent indicating different, potentially important, regulatory activities for this class of biomolecules outside the realm of DA signaling.

In light of the above, the lack of a well-characterized cell- or organ-based expression system made the difficulties of monitoring *de novo* incorporation of isotopically-labeled L-tyrosine (TYR), L-DOPA or DA into endogenous morphine appear insurmountable. Spector's group, however, made considerable advances in characterizing biosynthetic events involving *in vivo* enzymatic conversion of morphinan precursors into endogenous morphine, i.e., the later stages of the biosynthetic pathway. Key studies demonstrated stereoselective conversion of the morphinan alkaloids (+)-salutaridine, (–)-thebaine, and (–)-codeine into chemically authentic morphine in rat tissues [56] and transformation of thebaine to oripavine, codeine, and morphine by rat liver, kidney, and brain microsomes in the presence of NADPH- and NADH-generating systems [57]. Importantly, use of chemical inhibitors indicated a critical role of cytochrome P450 (CYP) isoenzymes in these synthetic processes [57].

Contemporaneously, Goldstein and coworkers reported the presence of morphine-like and codeine-like immunoreactivities in bovine hypothalamus and adrenal, and in rat brain, that were chemically characterized as authentic morphine

and codeine [58–60]. They proceeded to demonstrate *in vivo* and *in vitro* intramolecular conversion of reticuline to form salutaridine in rat liver, a critical step in generating the morphine/morphinan skeleton and the stereochemistry of the morphinan series [60]. Subsequent studies from Zenk and coworkers provided further characterization of hepatic conversion of reticuline to salutaridine [61,62], thereby reinforcing the critical involvement of CYPs in endogenous morphine expression.

2. Stereoselective formation of TIQ, BIQ alkaloid precursors: rejection of the long-held hypothesis of random, non-enzymatic, production of Pictet-Spengler condensation products

Neurochemical analysis of human brain has detected the presence of very low concentrations of only the biologically relevant (S) enantiomer of THP that is utilized in morphine biosynthesis, indicating enzyme-catalyzed expression of biosynthetic intermediates within a defined biosynthetic pathway [63]. The absence of racemic THP also dispels serious consideration of the hypothesis that this essential morphine precursor is formed via random non-enzymatic Pictet-Spengler condensation reactions of DA with 3,4-dihydroxyphenylacetaldehyde [7,8,18,30]. Similar considerations apply to the *in vivo* expression of non-racemic (R)-salsolinol formed enzymatically from DA and acetaldehyde in extracted human brain samples [25,32]. Demonstration of stereoselective expression of BIQ alkaloid precursors is complemented by later studies demonstrating the exclusive expression of the (S) enantiomer of the BIQ alkaloid morphine precursor (S)-reticuline in cultured SH-SY5Y human neuroblastoma and DAN-G human pancreatic carcinoma cells [64,65]. In these same analyses, ring-labeled (S)-THP, not (R), was stereoselectively incorporated into endogenous morphine via intramolecular isomerization of (S)- to (R)-reticuline. Recent work from our group has demonstrated an approximate 3-fold enhancement of tissue concentrations of endogenous morphine following administration of THP to an *ex vivo* preparation of invertebrate ganglia [16]. The observed rate of conversion of THP to morphine of approximately 20% when compared to the low steady-state levels of tissue THP suggests high intrinsic clearance of THP and other morphine precursors through a defined cellular biosynthetic pathway.

3. Contribution of recent *de novo* biosynthetic studies

Recent studies from our group employing well established *ex vivo* invertebrate nervous tissue preparations and primary cultures of human white blood cells [66–68] and those by Zenk and coworkers using human tumor-derived cell lines [64,65] have markedly contributed to our understanding of *de novo* morphine synthesis in animal systems and have facilitated the formulation of our evidence-based model. Key observations from these studies indicate that L-TYR, its monoamine homolog TA, and their respective catechol derivatives, L-DOPA and DA serve as substrates for *de novo* morphine and that pharmacological characterization of tyramine (TA) utilization as a morphine precursor indicates one or more catalytic steps mediated by microsomal CYP 2D6 [66,67]. These data are complemented by metabolic labeling/isotope enrichment studies employing SH-SY5Y neuroblastoma cells [64,65] indicating asymmetric isotopic labeling of the benzyl and isoquinoline chemical domains

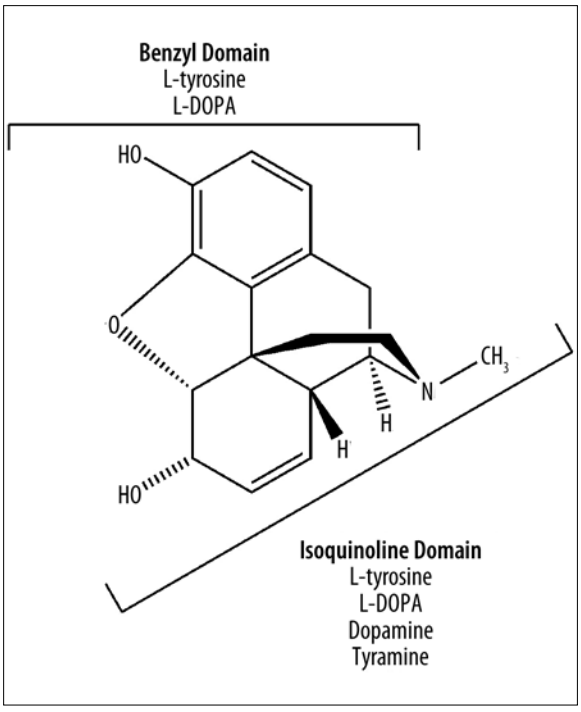

Figure 1. Schematic representation of *de novo* precursor incorporation into the morphine nucleus. L-TYR and L-DOPA are incorporated in both the benzyl and isoquinoline chemical domains of morphine, whereas DA and TA are only incorporated into the isoquinoline domain.

of newly formed morphine that is operationally determined by the type of L-TYR-derived precursor molecule that is employed. As schematically depicted in Figure 1, L-TYR and L-DOPA are incorporated in both the benzyl and isoquinoline chemical domains of morphine, whereas DA and TA are only incorporated into the isoquinoline domain.

These summary data effectively present a case for separate and distinct cellular pools of L-TYR-derived substrates targeted for *de novo* morphine synthesis in animal cells, and reject previously published biosynthetic schemes indicating that THP production is exclusively derived from DA [6–11,34]. THP formation involves enzymatic condensation and rearrangement of DA and 3,4-dihydroxyphenylacetaldehyde. Our formulated model establishes a *stoichiometric* relationship of one molecule of DA, derived from L-DOPA decarboxylase (DDC)-catalyzed decarboxylation of L-DOPA or CYP2D6-catalyzed ring hydroxylation of TA, to one molecule of 3,4-dihydroxyphenylacetaldehyde derived directly from L-DOPA without intermediate conversion to DA, thereby rejecting the long held hypothesis that monoamine oxidase (MAO) is a key enzyme involved in THP formation *in vivo* [9-11,13-15,29,30,34-37,69].

Recognition that enzyme-catalyzed THP formation represents a committed regulatory step in morphine biosynthesis by animal cells: analogies to (S)-Norcoclaurine synthase characterized in *Papaver somniferum*

A defined stoichiometric relationship of L-TYR-derived substrates from separate and distinct cellular pools has been de-

finitively established for morphine and related BIQ alkaloid synthesis in *Papaver somniferum* [70–73]. As depicted in Figure 2, (S)-Norcoclaurine synthase (EC 4.2.1.78) catalyzes the stereoselective condensation and rearrangement of DA and 4-hydroxyphenylacetaldehyde to form (S)-norcoclaurine as the first committed step in the biosynthesis of BIQ alkaloids including morphine, sanguinarine, and berberine [73]. In the plant pathway, DA is produced by decarboxylation of L-DOPA via the action of pyridoxal phosphate-dependent progenitor isoenzymes with dual TYR decarboxylase (TDC) and DDC activities [70–72]. 4-hydroxyphenylacetaldehyde is derived from TA produced by decarboxylation of L-TYR mediated by the same progenitor TDC/DDC isoenzymes. The plant equivalents of mammalian CYPs/monooxygenases mediating ring hydroxylation of L-TYR and TA to form L-DOPA and DA, respectively, have not been identified. Furthermore, it has not been determined whether conversion of TA to 4-hydroxyphenylacetaldehyde is effected by transamination or decarboxylation of L-TYR coupled to oxidative deamination (discussed below).

The functional association of pyridoxal phosphate-dependent decarboxylases in opium poppy with mammalian DDC in the mediation of morphine biosynthesis in animal systems is compelling. Accumulated biochemical data indicate that in addition to its well characterized decarboxylation of L-aromatic amino acids, as a side reaction mammalian DDC catalyzes a decarboxylation-dependent transamination or oxidative deamination of aromatic amines, effectively converting L-DOPA into 3,4-dihydroxyphenylacetaldehyde with simultaneous conversion of enzyme-bound pyridoxal phosphate into pyridoxamine phosphate [74–78]. The reaction specificity of DDC toward aromatic amines depends on the experimental conditions: oxidative deamination occuring under aerobic, half-transamination occurring under anaerobic conditions.

A model of THP formation is schematically depicted in Figure 3 that represents a best fit, evidence-based, formulation of the first committed step in cellular morphine production by animal cells. A key aspect of the model is the establishment of a *stoichiometric* relationship of DA derived from DDC-catalyzed decarboxylation of L-DOPA or CYP2D6-catalyzed ring hydroxylation of TA to 3,4-dihydroxyphenylacetaldehyde derived directly from L-DOPA via DDC-mediated oxidative deamination or half-transamination. The proposed mechanism also requires recognition, identification, and biochemical characterization of a regulatory enzyme with (S)-Norlaudanosoline synthase activity corresponding to (S)-Norcoclaurine synthase in plants. In support of these contentions, previous biochemical studies have provided initial characterization of salsolinol synthase, the enzyme responsible for catalyzing the committed step in the formation of the TIQ alkaloid (R)-salsolinol *in vivo* [25-32]. As depicted in Figure 4, Salsolinol synthase enzymatically condenses and rearranges one molecule of DA and one molecule of acetaldehyde or pyruvic acid to form either (R)-salsolinol or salsolinol-1-carboxylic acid, respectively. Salsolinol-1-carboxylic acid is non-enzymatically converted to (R)-salsolinol. Biochemical studies to determine whether Salsolinol synthase also possesses significant S)-Norlaudanosoline synthase activity has not yet been performed. Convergent presumptive evidence, however, gleaned from biochemical studies of (S)-Norcoclaurine synthase in plants and Salsolinol synthase in mammalian systems provide strong support for the existence of (S)-Norlaudanosoline synthase as the committed enzyme responsible for THP formation leading to endogenous morphine expression by animal cells.

(S)-Norlaudanosoline synthase catalyzes the stereoselective condensation and rearrangement of DA and 3,4-dihydroxyphenylacetaldehyde to form (S)-THP as the first committed step in the biosynthesis of endogenous morphine (Figure 3). The significance of TA as a biosynthetic intermediate is validated by *in vitro* enzyme kinetic studies demonstrating DA formation via CYP 2D6-catalyzed ring hydroxylation of TA [79–82] which in turn lends support to the existence of a potentially important tyrosine hydroxylase (TH)-independent pool of cytosolic DA that is available for endogenous morphine expression [66-68]. TA has been established as genuine transmitter with distinct biological functions in the nervous systems of animals ranging in complexity from Caenorhabditis elegans to mammals [83-89]. These recent data indicating TA as an essential BIQ precursor at the early stages of morphine biosynthesis reinforce its biological significance in higher animal systems and also highlight the important role of CYP2D6 to mediate its *in vivo* conversion to DA. The involvement CYP2D6 at later stages of morphine biosynthesis is supported by previous biochemical studies that have characterized its O-demethylation activity towards codeine and related opiate alkaloids [90].

5. Transamination of L-TYR and L-DOPA as a major regulatory mechanism responsible for compartmentalization and mobilization of essential substrate pools required for endogenous morphine biosynthesis

A compelling model of *de novo* morphine biosynthesis in animals must include regulatory mechanisms responsible for the compartmentalization and mobilization of essential substrate pools of L-TYR and L-TYR-derived molecules targeted for BIQ alkaloid production. We propose reversible transamination of L-TYR and/or L-DOPA via pyruvic acid intermediates as a major regulatory mechanism responsible for cellular sorting and/or functional sequestration of substrate pools of L-TYR-derived molecules targeted for endogenous morphine production. These contentions are supported by *in vivo* pharamacological studies performed in rats indicating that D-3,4-dihydroxyphenylalanine (D-DOPA) and its familiar racemate L-DOPA are capable of producing DA-related behavioral effects with similar potency and that D-DOPA and 3,4-dihydroxyphenylpyruvate when infused into the rat striatum significantly increase extracellular DA levels [91–95].

In vivo synthesis of DA from D-DOPA is hypothesized to proceed by the following mechanism: transamination/oxidation of D-DOPA to form the alpha-keto acid intermediate 3,4-dihydroxyphenylpyruvate, a second transamination to convert 3,4-dihydroxyphenylpyruvate to L-DOPA, followed by DDC-catalyzed decarboxylation to DA. A subsequent study demonstrated that 3,4-dihydroxyphenylpyruvate is an effective DDC inhibitor and proposed that its significant L-DOPA sparing effects are mediated by its participation in transamination events and by its inhibition of decarboxylation [96].

Complementary studies have also provided initial biochemical characterization of transamination of L-TYR, L-DOPA or L-tryptophan in the presence of phenylpyruvate and al-

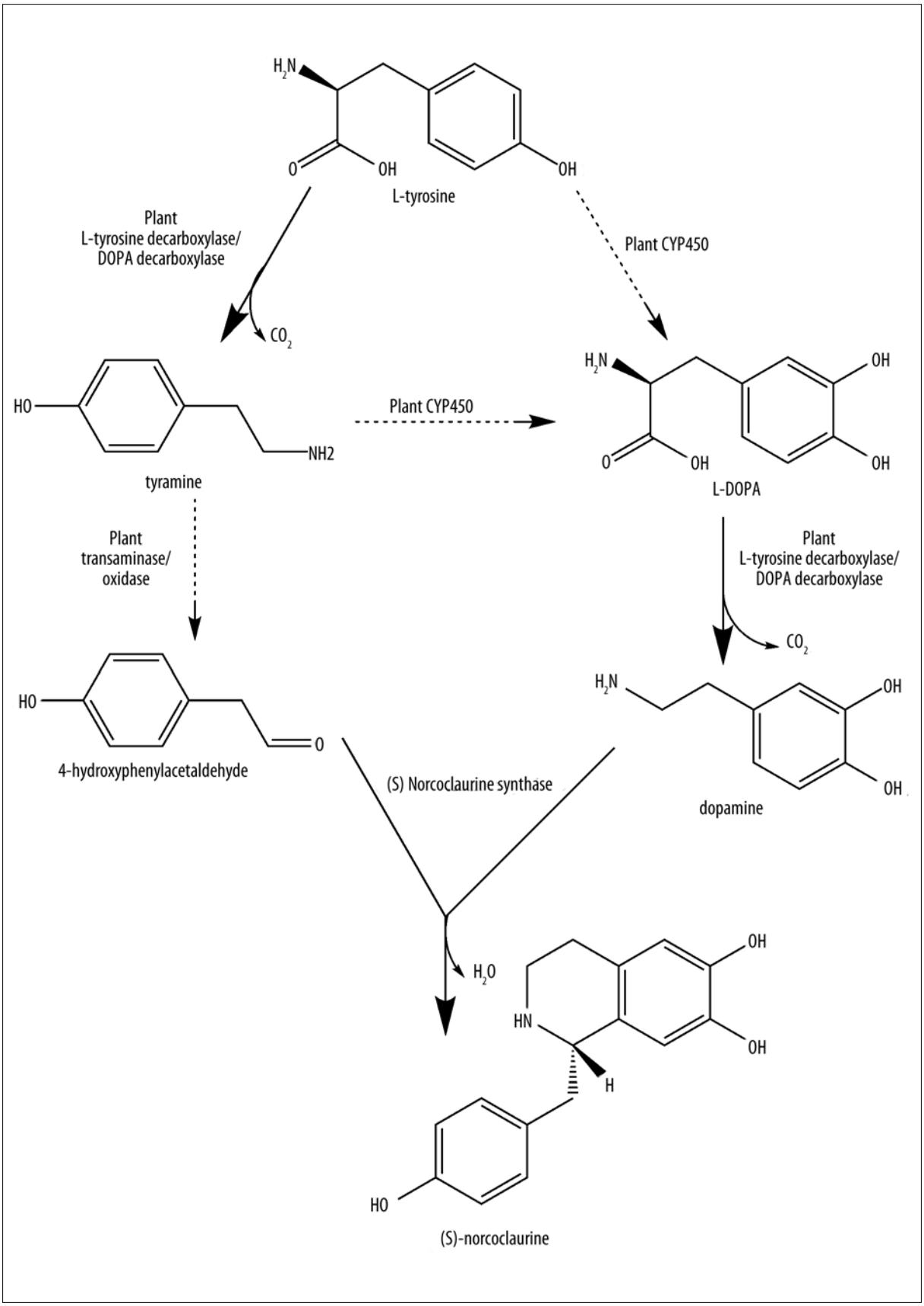

Figure 2. Norcoclaurine synthase-catalyzed formation of (S)-norcoclaurine in *Papaver somniferum*. Norcoclaurine synthase (EC 4.2.1.78) catalyzes the stereoselective condensation and rearrangement of DA and 4-hydroxyphenylacetaldehyde to form (S)-norcoclaurine as the first committed step in the biosynthesis of BIQ alkaloids. Broken arrows indicate enzyme steps awaiting further biochemical elucidation.

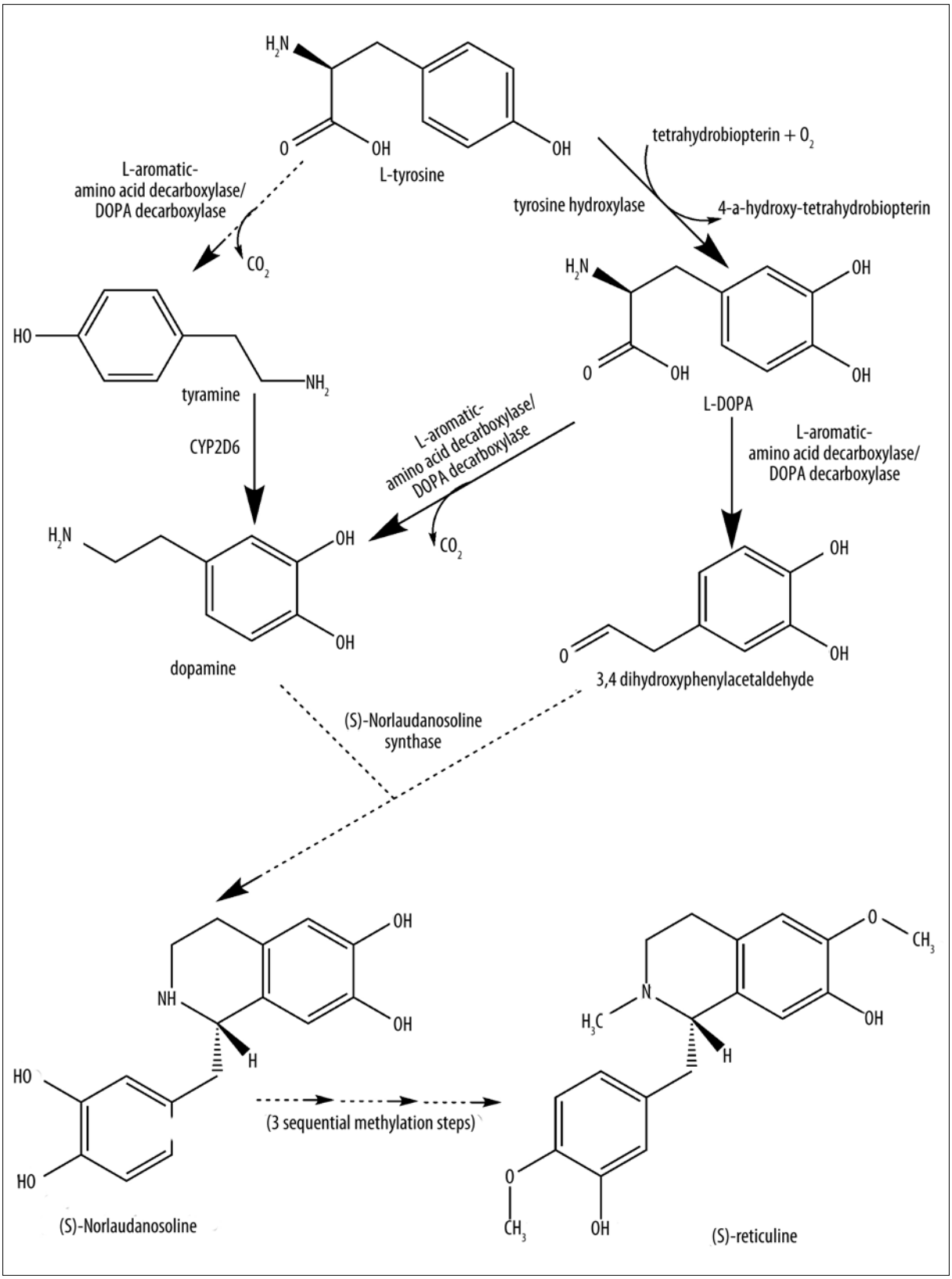

Figure 3. Evidence-based model of (S)-THP formation and conversion to (S)-reticuline: formulation of the first committed step in cellular *de novo* morphine production by animal cells. The model establishes a *stoichiometric* relationship of DA derived from DDC-catalyzed decarboxylation of L-DOPA or CYP2D6-catalyzed ring hydroxylation of TA to 3,4-dihydroxyphenylacetaldehyde derived directly from L-DOPA via DDC-mediated oxidative deamination or half-transamination. Our proposed mechanism also requires recognition, identification, and biochemical characterization of a regulatory enzyme with (S)-Norlaudanosoline synthase activity corresponding to (S)-Norcoclaurine synthase in plants. Broken arrows indicate enzyme steps awaiting further biochemical elucidation.

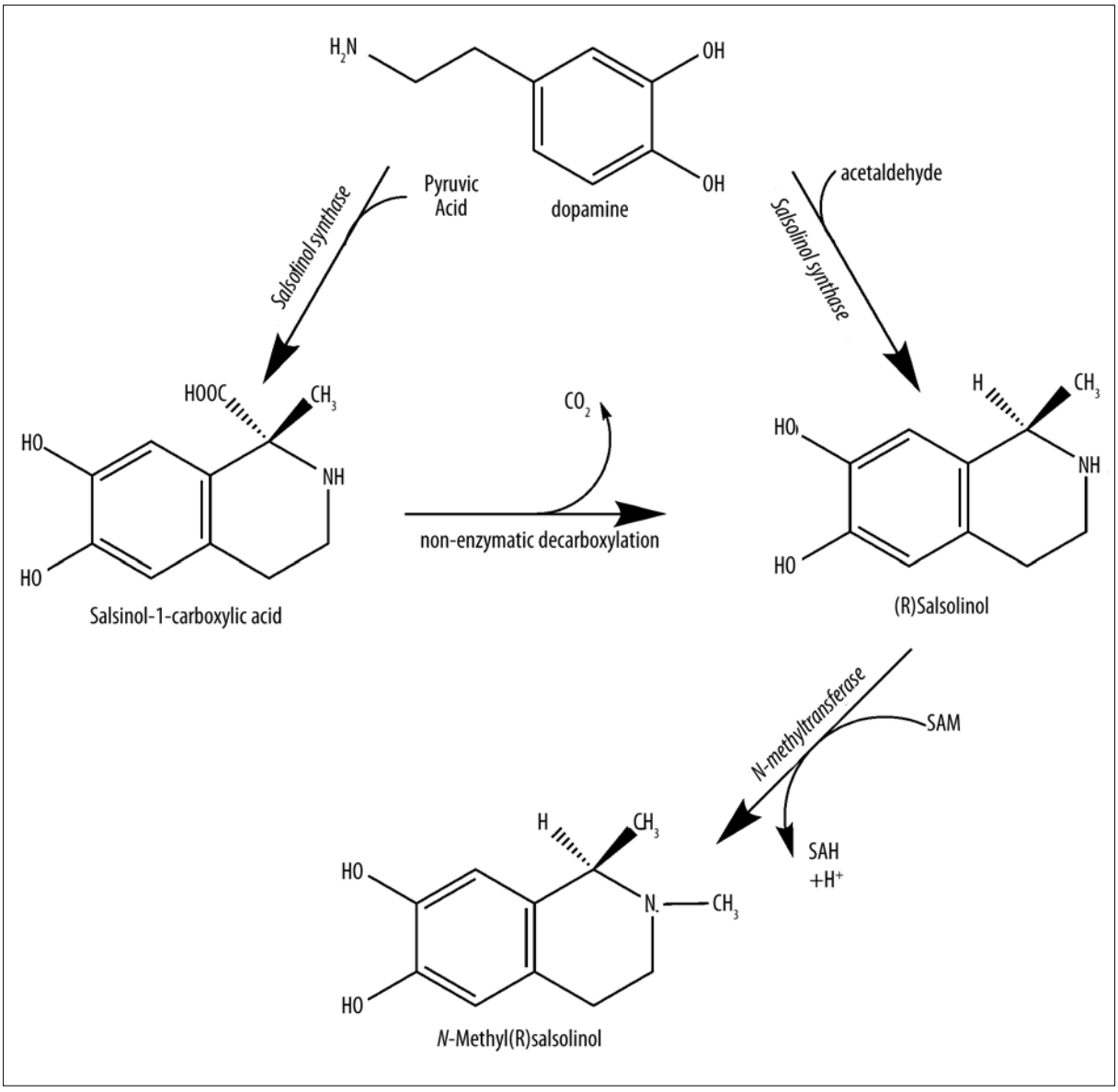

Figure 4. Salsolinol synthase-catalyzed formation of (R)-salsolinol in animal cells. Salsolinol synthase enzymatically condenses and rearranges one molecule of DA and one molecule of acetaldehyde or pyruvic acid to form either (R)-salsolinol or salsolinol-1-carboxylic acid, respectively. Salsolinol-1-carboxylic acid is non-enzymatically converted to (R)-salsolinol.

pha-ketoglutarate as co-substrates [97]. L-TYR and L-DOPA aminotransferase activities with phenylpyruvate as a co-substrate were determined to be functionally distinct from well-characterized aminotransferases utilizing alpha-ketoglutarate as the acceptor keto acid [97]. Identification of cellular aminotransferases utilizing aromatic amino acids and aromatic alpha-keto acids as co-substrates provides additional mechanistic evidence for the functional inter-conversion and/or sequestration of L-TYR and L-TYR-derived substrate pools targeted for morphine biosynthesis.

6. An alternate evidence-based model of *de novo* morphine biosynthesis

The discovery and initial pharmacological characterization of norlaudanosolinecarboxylic acids, including 3'O-methyl-norlaudanosolinecarboxylic acid, presumably representing the condensation products of DA and 3,4-dihydroxyphenylpyruvate [98], provide us with provocative evidence in support of an alternate pathway of *de novo* morphine biosynthesis (Figure 5). The proposed alternate biosynthetic pathway involves (S)-Norlaudanosoline synthase-catalyzed stereoselective condensation and rearrangement of DA and the transmination product of L-DOPA, i.e., 3, 4-dihydroxyphenylpyruvate, to form (S)-norlaudanosoline-1-carboxylic acid. (S)-norlaudanosoline-1-carboxylic acid is non-ezymatically decarboxylated to form THP, in similar fashion to the formation of (R)-salsolinol from salsolinol-1-carboxylic acid depicted in Figure 4. Formation of THP directly from 3,4-dihydroxyphenylpyruvate also reinforces the importance of transamination of aromatic amino acids/aromatic alpha-keto acids as a major regulatory mechanism in the expression of endogenous morphine and extends the biosynthetic definitions of appropriate L-TYR-derived substrates that may be utilized in

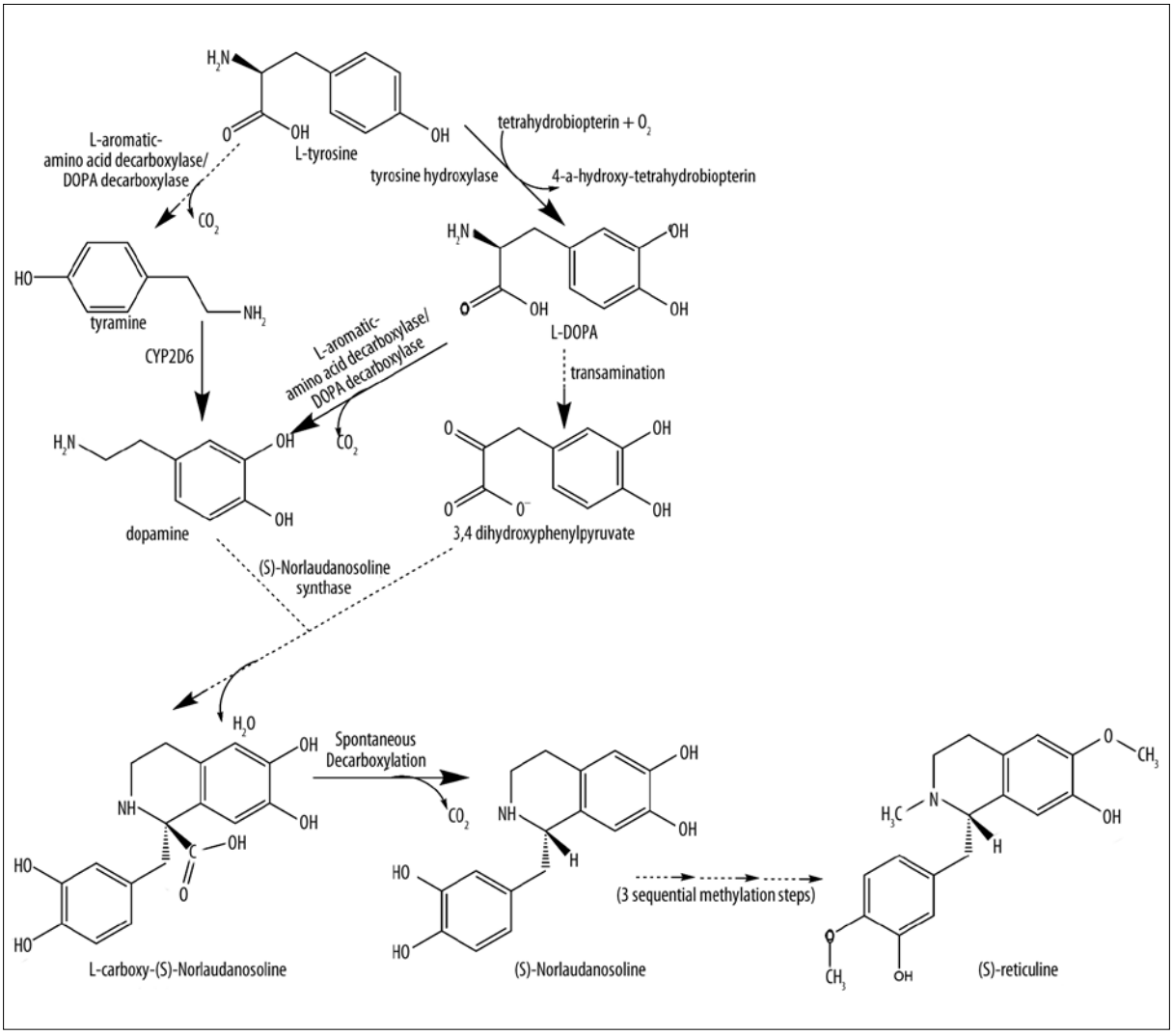

Figure 5. Evidence-based model of (S)-THP formation and conversion to (S)-reticuline: formulation of an alternate biosynthetic pathway. The alternate model involves (S)-Norlaudanosoline synthase-catalyzed stereoselective condensation and rearrangement of DA and the transmination product of L-DOPA, i.e., 3, 4-dihydroxyphenylpyruvate, to form (S)-norlaudanosoline-1-carboxylic acid. (S)-norlaudanosoline-1-carboxylic acid is non-ezymatically decarboxylated to form THP, in similar fashion to the formation of (R)-salsolinol from salsolinol-1-carboxylic acid depicted in Figure 4.

these processes. Interestingly, pharmacological characterization of norlaudanosolinecarboxylic acids as potent inhibitors of hepatic catechol-O-methyltransferase (COMT) at physiologically relevant micrommolar concentrations also provides us with additional insight into the biosynthetic mechanisms responsible for *in vivo* conversion of THP into the essential morphine precursor (S)-reticuline, as definitively established in plants [99–102] (Figure 6). The existence of steady state levels of 3'O-methylnorlaudanosolinecarboxylic acid [103] lends support to its biological role as a precursor to 3'O-methylnorlaudanosoline that later converted to (S)-reticuline via sequential N- and O-methylation events.

7. Putative cellular regulatory activities of endogenous morphine: extensions of the evidence-based model

Strengthening the endogenous morphine presence and synthesis in animal tissues are the pharmacological data, demonstrating the specific manipulation of the system in a manner consistent with regulation. The CYP2D6 inhibitor, quinidine [104], in a concentration dependent manner, decreased Mytilus ganglionic morphine levels when ganglia were exposed to tyramine as did AMPT, a tyrosine hydroxylase inhibitor, when ganglia were exposed to tyrosine [66]. Exposure to either enzyme inhibitor alone did not significantly reduce morphine levels below the level of non-exposed ganglia [66]. However, exposure of pedal ganglia to both enzyme inhibitors did reduce ganglionic morphine levels significantly, demonstrating compensatory effects between both pathways, here operating simultaneously.

The significance of CYP2D6 in ganglionic morphine biosynthesis was demonstrated in the steps from TA to DA and THP to codeine, which resulted in a significant blocked by quinidine [66]. These experiments demonstrated that CYP2D6 is involved with morphine synthesis, which was supported by RT-PCR analysis amplifying a 282 bp fragment, demonstrating the presence of CYP2D6 mRNA [104]. Sequence

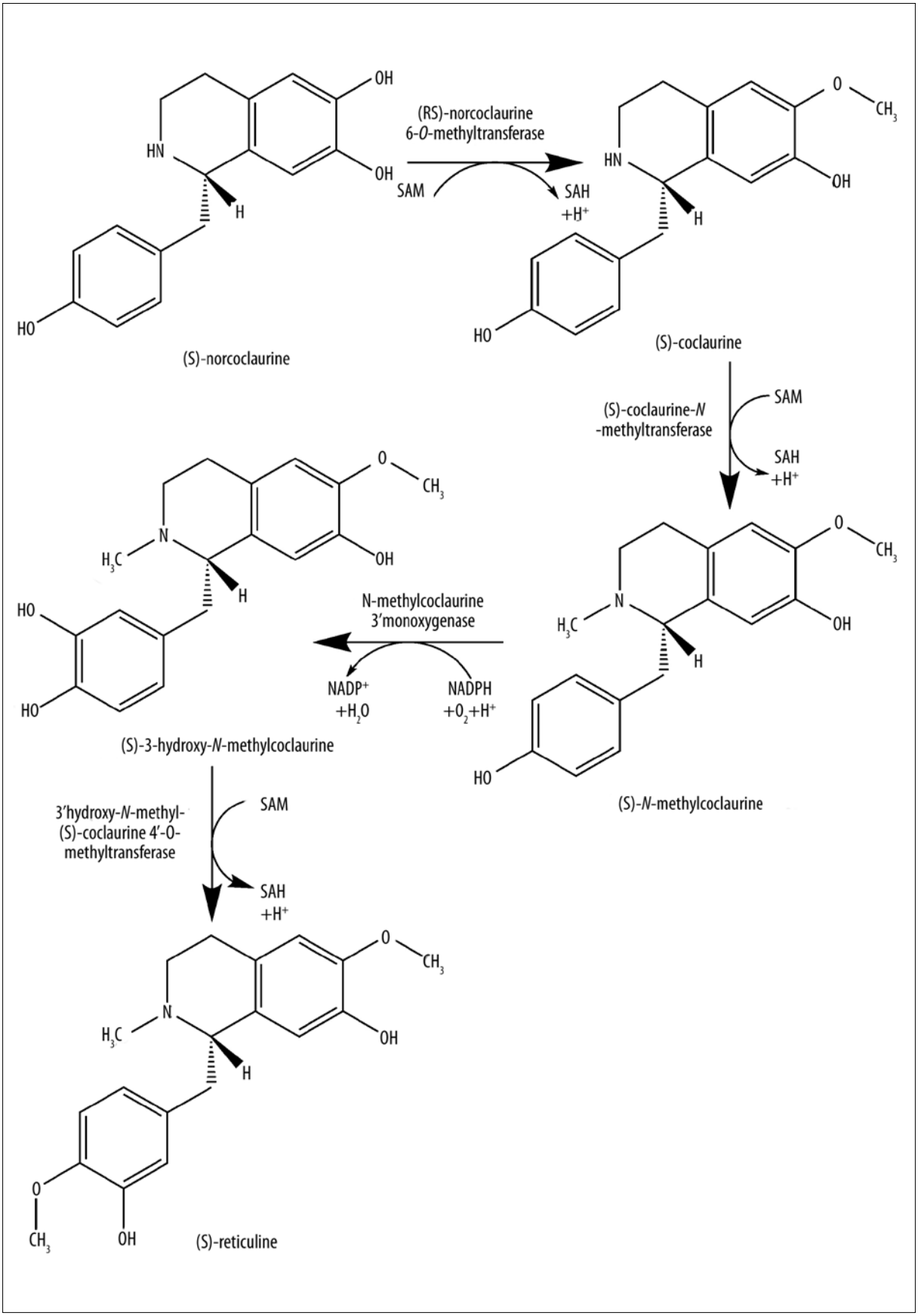

Figure 6. Biosythetic pathway of (S)-reticuline formation from (S)-norcoclaurine in *plants*. (S)-norcoclaurine is converted to (S)-reticuline by sequential O- and N-methylation events and an additional ring hydroxylation, as described in references 99-102.

analysis of this transcript fragment demonstrated a 94% similarity to human GeneBank accession number M20403 in the invertebrate tissue [66]. Furthermore, normal, human white blood cells (WBC), specifically polymorphonuclear cells (PMN), contain and have the ability to synthesize morphine also via CYP2D6 as noted by its inhibition by quinidine [67]. These studies provide evidence that: (1) the synthesis of morphine by various animal tissues is more widespread than previously thought and now includes human immune cells; (2) Moreover, another pathway for morphine synthesis exists, via L-DOPA, demonstrating an intersection between dopamine and morphine pathways; (3) White blood cells can release morphine into the environment to regulate themselves and other cells. Therefore, white blood cells employ endogenous expressed morphine as a key autocrine/paracrine signaling factor.

Previous studies from our laboratory have revealed a novel μ opiate receptor, μ_3, which is expressed in different animal tissues such as human vascular endothelial cells, leukocytes and neural tissues [105]. This novel mu receptor is selective for the opiate alkaloid morphine since opioid peptides do not bind to this mu-type splice variant receptor. In reporting on the acute affects of morphine exposure to human leukocytes by analyzing the expression of different genes it was revealed that exogenously applied morphine down regulated TH expression, suggesting an end-product inhibition mechanism modulating the pathway of morphine biosynthesis [106]. In the same study it was demonstrated that constitutive nitric oxide synthase (cNOS) was up-regulated and inducible NOS was down regulated, confirming our previous observations [53,105]. We have demonstrated that μ_3 via morphine activation releases cNOS in immune, vascular, gut and neural tissues see [1,107]. In this regard, NO also inhibits dopamine β hydroxylase (see [108]), suggesting that via NO the morphine biosynthetic pathway is favored by cutting off further catecholamine synthesis and diverting the extra DA to morphine synthesis.

Taken together, we have surmised that the low concentrations of morphine found in various tissues serve to limit their excitability, micro environmental noise [109], which upon trauma, after a latency period, increase in an attempt to again limit their excitability [2,3]. This suggests that the respective tissues are always in the "on" state, allowing them to emerge immediately from this diminished excitability state and spring into action, which is critical for survival, and after a period of time they are brought back into the down state via cNOS [2,53,109]. This hypothesis is also supported by a microarray study of human genes, whereby morphine exposure to PMN down regulated proinflammatory mediator expression [106]. Recently, we have also demonstrated that exogenous morphine exposure can have positive implications for Alzheimer's and Parkinson's disorders [10,110]. We have even demonstrated that morphine may be a key component of relaxation associated with various human activities [4,111–113]. The fact that this signaling system is present in organisms 500 million years divergent in evolution, performing identical functions, except those involved with, also supports a protective role for morphine.

The *de novo* biosynthesis of morphine in animal cells provides us with an expanded set of cellular processes that require BIQs as defined biosynthetic intermediates/enzyme substrates, alternative explanations of the biological relevance of isoquinoline alkaloids over and beyond those linked to DA-induced cellular toxicity, and establishes an underlying chemical basis for phylogenic conservation and adaptation of reciprocally interactive catecholamine and opioid signaling pathways.

Adaptive nociceptive, nocifensive, and anti-nociceptive behaviors have evolved from paracrine cellular processes. As a prime example, in *Papaver somniferum*, pyridoxal phosphate-dependent progenitor isoenzymes with dual TDC and DDC activities as well as berberine bridge enzyme, i.e., key players in the biosynthesis of BIQ alkaloids [65,70,71], are induced following traumatic insult to opium poppy cells via activation of wound-responsive regulatory elements on their respective genes [114]. The differential expression of these essential gene products and the organ-dependent accumulation of different alkaloids suggest a coordinated regulation of specific alkaloid biosynthetic genes. Accordingly, we propose that positive evolutionary pressure that has preserved a primordial, phylogenic broad, biochemical mechanism by which the prototype opiate alkaloid morphine is expressed and utilized as a physiological regulator of relatively simple as well as significantly more complex cellular functions [2].

Recent findings by Goumon et al, also demonstrate that endogenous morphine 6 glucuronide is synthesized in chromaffin cells and secreted into the incubation medium upon stimulation [115]. This finding strongly suggests that this material may be released from adrenal tissues in response to stressors. In the peripheral circulation, morphine-6-glucuronide may mediate several systemic actions (e.g. on immune cells) based on its affinity for the μ_3 opiate receptor. In sum, these data represent an important observation on the role of morphine-6-glucuronide as a new endocrine factor, which may be released from adrenal tissues in response to stressors, thereby mediating neural-immune coupling events [116-118].

EPILOGUE

Throughout the 1970s, considerable cross-fertilization of ideas and hypotheses between alcohol abuse and Parkinson's Disease researchers into the origin and biological significance of isoquinoline alkaloids resulted in a historical convergence of numerous studies attempting to define DA-derived TIQ and BIQ alkaloids as addictive agents responsible for alcohol dependence and as major neurotoxic agents responsible for the etiology and persistence of Parkinson's Disease. Importantly, the demonstration of *in vivo* conversion of THP to morphine provides invaluable mechanistic insight into previous pharmacological studies involved with focal administration of THP into DA-ergic limbic areas associated with ethanol abuse [12,18,20], supports the critical involvement of morphine-preferring mu opioid receptors (MORs) in limbic areas associated with ethanol intake behaviors [113,119–121], and adds an additional dimension to alcohol abuse research using both pharmacological inhibitors of morphine biosynthesis as well as type selective opioid antagonists [122–124].

Highlighting the importance of endogenous morphine are recent studies demonstrating that addictive properties of nicotine, alcohol and cocaine may arise from their ability to

enhance endogenous morphine levels and its neuronal release, opening up a new level of understanding in substance abuse induced addiction and behavioral effects, as well as morphine regulation [12,16]. In the past, these substances of abuse have again been linked into a common pathway because of the common DA connection [125,126]. Now, they are additionally linked because of their common effect on morphinergic processes. It is highly significant that both nicotine and ethanol increase ganglionic morphine levels rapidly, providing a mechanism to initiate their pleasure and addicting actions with continued frequent use.

CONCLUSIONS

In conclusion, scientific orthodoxy has attempted to establish rational guidelines by which it may construct an empirically-driven, consistently tame, superstructure to encompass cellular regulation of complex biological processes in higher organisms. As a corollary, the collective effort to codify general principles of cellular organization has effectively resulted in the marginalization of many important lines of empirical investigation that are perceived to inject varying degrees of disorder and/or controversy into well-ordered regulatory schemes. A prime example, the biochemical and physiological investigation into the expression and functional roles of endogenous morphine by animal cells has presented an ongoing challenge to several research groups for over thirty years. A compelling body of evidence now supports the existence of a *de novo* biosynthetic pathway for endogenous morphine in mammalian and invertebrate cells, with remarkable similarities to the well-characterized enzymatic pathway described in *Papaver somniferum*. Elucidation of the potential biological significance/impact of evolutionarily conserved opiate alkaloid plant products in animal cells awaits further investigation [127].

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