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Tyrosine and tyramine increase endogenous ganglionic morphine and dopamine levels *in vitro* and *in vivo*: cyp2d6 and tyrosine hydroxylase modulation demonstrates a dopamine coupling

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: The ability of animals to make morphine has been in question for the last 30 years. Studies have demonstrated that animals do contain morphine precursors and metabolites, as well as the ability to use some morphine precursors to make morphine.

Material/Methods: The present study uses excised ganglia from the marine invertebrate *Mytilus edulis* as well as whole animals. Morphine and dopamine levels were determined by high performance liquid chromatography coupled to electrochemical detection and radioimmunoassay. Tissues and whole animals were also exposed to morphine precursors and exposed to the CYP2D6 inhibitor quinidine and the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT). Additionally, via RT-PCR, a cDNA fragment of the CYP2D6 enzyme in the ganglia of *M. edulis* was identified.

Results: Pedal ganglia incubated with either tyramine or tyrosine, or whole animals receiving injections, exhibited a statistically significant concentration- and time-dependent increase in their endogenous morphine and dopamine levels (2.51±0.76 ng/g for tyrosine and 2.39±0.64 ng/g for tyramine compared to approximately 1.0 ng/g morphine wet weight). Incubation with quinidine and/or AMPT diminished ganglionic morphine and dopamine synthesis at various steps in the synthesis process. We also demonstrated that CYP2D6 mediates the tyramine to dopamine step in this process, as did tyrosine hydroxylase in the step from tyrosine to L-DOPA. Furthermore, via RT-PCR, we identified a cDNA fragment of the CYP2D6 enzyme in the ganglia, which exhibits 94% sequence identity with its human counterpart. Evidence that tyrosine and tyramine were, in part, being converted to dopamine then morphine, and that this process can be inhibited by altering either or both CYP2D6 or tyrosine hydroxylase, is also provided.

Conclusions: It appears that animals have the ability to make morphine. This process also appears to be dynamic in that the inhibition of one pathway allows the other to continue with morphine synthesis. Moreover, dopamine and morphine synthesis were coupled.

key words: **morphine • tyrosine • tyramine • nervous tissue • invertebrates ganglia • endogenous morphine • CYP2D6 • alpha-methyl-para-tyrosine • dopamine • tyrosine hydroxylase**

Abbreviations: **HPLC – High performance liquid chromatography; THP – tetrahydropapoverine/norlaudanosoline; PBS – phosphate buffered saline; RIA – radioimmunoassay; AMPT – alpha-methyl-para-tyrosine; DA – dopamine**

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BACKGROUND

Many studies have supported the hypothesis that normal animal tissues have the ability to make morphine. There is a body of evidence demonstrating that morphine, morphine-3- and 6- glucuronide, as well as morphine putative precursor molecules (thebaine, salutaridine, norcocolarine, reticuline and codeine) exist in vertebrates [1–4] and in invertebrates [5–10]. Furthermore, exposing healthy *Mytilus edulis* pedal ganglia to reticuline or L-DOPA increases endogenous ganglionic morphine levels [11,12]. These data provide important evidence, supporting the hypothesis, that animals have the ability to synthesize morphine.

In the present report we extend these observations to include tyrosine, and the amino acid metabolite tyramine, which give rise to the previously mentioned morphine precursors. We demonstrate that tyrosine and tyramine also have the ability to augment endogenous ganglionic morphine and dopamine levels in *M. edulis* under *in vitro* and *in vivo* experimental protocols. Additionally, this process is in part regulated by CYP2D6, a cytochrome P450 isoform, and tyrosine hydroxylase, dynamically coupled to ensure morphine synthesis occurs even if one pathway is inhibited.

MATERIAL AND METHODS

M. edulis collected from the local waters of Long Island Sound were initially scrubbed and washed to remove any materials on their shells and maintained as previously described in detail [13]. Briefly, they were maintained in 0.22 µm filtered artificial seawater (30 ppt salts) at 18°C (Instant Ocean, Aquarium Systems, Mentor, OH) for one month prior to experimentation. The aquarium used UV light and a protein skimmer as well as a biological filter to remove nitrogenous and other metabolic wastes. For the biochemical *in vitro* analysis of either dopamine (DA) or morphine, groups of 20 animals had their pedal ganglia excised on ice at different time periods and incubated with different amounts of tyrosine or tyramine, ranging from 1 to 100 ng. Ganglia were maintained in 1 ml of 0.22 µm filtered artificial sea water. Additionally, pedal ganglia were incubated with and without these precursors in the presence of quinidine, a CYP2D6 inhibitor [14], and alpha-methyl-para-tyrosine (AMPT), which inhibits tyrosine hydroxylase [15].

For *in vivo* treatments the animal's foot (20 animals per treatment) was injected with 100 µg of either tyramine or tyrosine dissolved in 100 µl of saline solution. Other animals were exposed to the enzyme inhibitors (AMPT or quinidine) alone or in combination immediately following the respective foot injection. After 1 hour incubation in seawater at room temperature, ganglionic morphine levels were determined. All chemicals were purchased from Sigma (St. Louis, MO).

Morphine determination, solid phase extraction

The morphine extraction protocol was performed in pooled ganglia as reported in detail elsewhere [8,9,11,16]. The dried extract was then dissolved in 0.05% trifluoroacetic acid (TFA) water before solid phase extraction. Samples were loaded on a Waters Sep-Pak Plus C-18 cartridge previously activated with 100% acetonitrile and washed with 0.05% TFA-water. Morphine elution was performed with a 10% acetonitrile solution (water/acetonitrile/TFA, 89.5%: 10%: 0.05%, v/v/v). The eluted sample was dried with a Centrivap Console and dissolved in water prior to high performane liquid chromatography (HPLC) analysis.

Radioimmuno-assay (RIA) determination

The morphine RIA determination is a solid phase, quantitative RIA, wherein ^{125}I-labeled morphine competes for a fixed time with morphine in the test sample for the antibody binding site. The commercial kit employed is from Diagnostic Products Corporation (USA) [4,8,9,11,16]. The detection limit was 0.5 ng/ml.

HPLC and electrochemical detection of morphine in the sample

The HPLC analyses were performed with a Waters 626 pump (Waters, Milford, MA) and a C-18 Unijet microbore column (BAS). A flow splitter (BAS) was used to provide the low volumetric flow-rates required for the microbore column. The split ratio was 1/9. Operating the pump at 0.5 ml/min, yielded a microbore column flow-rate of 50 µl/min. The injection volume was 5 µl. Morphine detection was performed with an amperometric detector LC-4C (BAS, West Lafayette, Indiana). The microbore column was coupled directly to the detector cell to minimize the dead volume. The electrochemical detection system used a glassy carbon-working electrode (3 mm) and a 0.02 Hz filter (500mV; range 10 nA). The cell volume was reduced by a 16-µm gasket. The chromatographic system was controlled by Waters Millennium^{32} Chromatography Manager V3.2 software and the chromatograms were integrated with Chromatograph software (Waters).

Morphine was quantified in the tissues by the method described by Zhu and Stefano [8]. Several HPLC purifications were performed between each sample to prevent residual morphine contamination remaining on the column. Furthermore, mantle tissue was run as a negative control, demonstrating a lack of contamination. All solutions devoid of animals or their tissues were also examined for any detectable morphine and none was found.

Extraction and HPLC UV detection of dopamine

Dopamine (DA) was extracted from both ganglia (20 pedal ganglia per treatment replicated 4 times) and hemolymph (10 ml hemolymph per treatment replicated 4 times). After ganglionic dissection, ganglia were pooled into one Eppendorf tube, added with 1 ml of 1 N HCl, and sonicated with a 60 sonic dismembrator (Fisher Scientific, USA). Homogenized tissue was then transferred to 15 ml polypropylene centrifuge tube (Fisher Scientific, PA, USA). 5 ml of chloroform/isopropanol (9:1, v/v) was added and vigorously vortexed for 5 min at room temperature. Tubes were centrifuged at 4000 rpm for 15 min at 4°C. Supernatant (water soluble layer) was dispatched into pre-siliconized 1.5 ml tubes (Midwest Scientific) and kept at 4°C for immediate use for HPLC determination or saved under –80°C for further analysis.

HPLC was performed with Waters 626 pump and 2487 Dual λ Absorbance Detector. An Xterra RP18 column with 5 µm

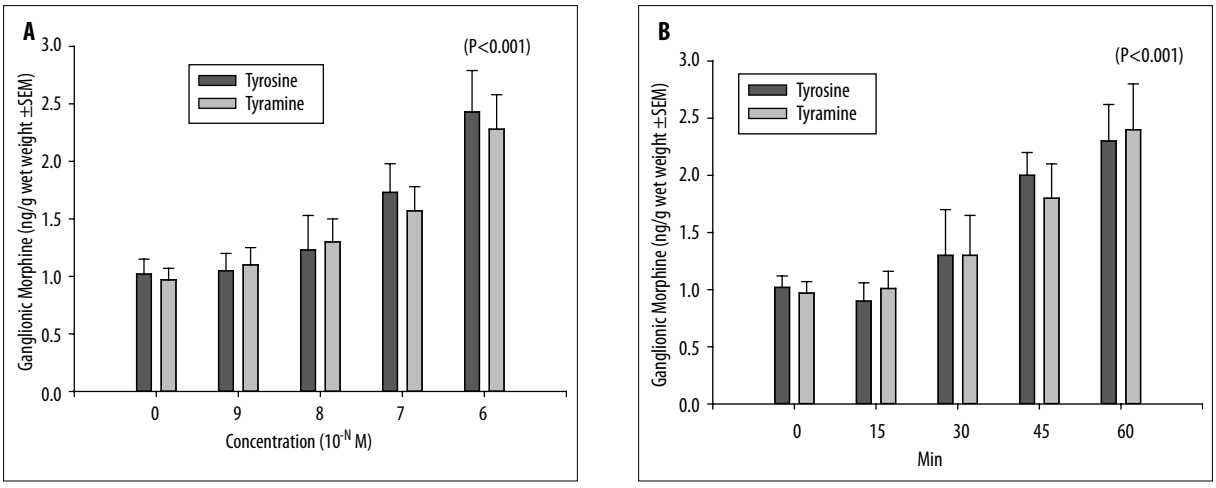


Figure 1. Pedal ganglia (20 per treatment) were incubated with the morphine precursor's tyrosine or tyramine at varying concentrations for varying time periods. (**A**) Ganglionic morphine levels significantly increased in a concentration dependent manner during the incubation. (**B**) Ganglionic morphine levels rose when compared to non-treated controls (P<0.001, One Way ANOVA) over time when incubated with the respective precursors (10^{-6} M shown). Each experiment was repeated 3 times and the mean plus/minus the SEM graphed. At concentrations of 10^{-7} and 10^{-6} M and at 45 and 60 min into the incubation these mean values were statistically significant at the P<0.05 level of confidence. There is no difference between saline and non-treated ganglia.

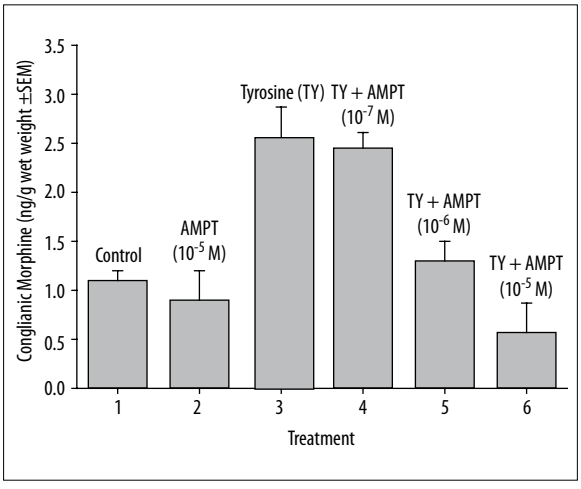


Figure 2. Tyrosine hydroxylase modulation of ganglionic morphine levels. Ganglia were incubated with tyrosine (10^{-6} M), and AMPT (alpha-methyl-para-tyrosine), a tyrosine hydroxylase inhibitor. The various treatments are as follows: **1.** Control Ganglia left undisturbed for 1 hour; **2.** Ganglia incubated with AMPT (10^{-5} M) alone; **3.** Ganglia incubated with tyrosine (10^{-6} M), which significantly enhanced morphine levels (P<0.001, One Way ANOVA); **4–6.** Ganglia incubated with tyrosine (10^{-6} M) plus AMPT at different concentrations. AMPT significantly diminished morphine levels in a concentration dependent manner compared to tyrosine alone (P<0.001, One Way ANOVA). Each experiment was repeated 4 times and the mean plus/minus the SEM graphed.

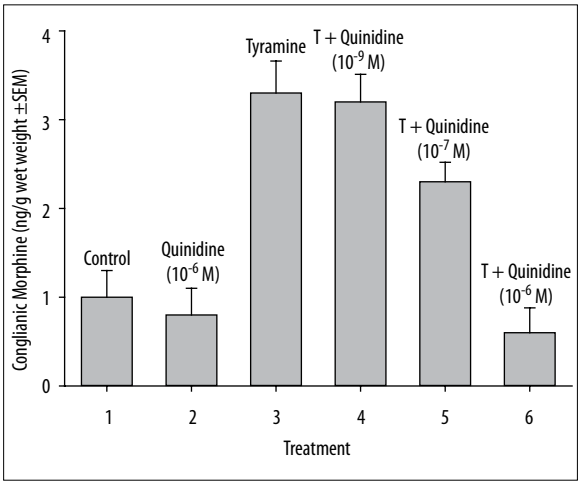


Figure 3. Ganglia incubated with the CYP2D6 specific inhibitor quinidine inhibited tyramine (T) stimulated morphine synthesis in *M. edulis* pedal ganglia. In a concentration dependent manner quinidine inhibited tyramine (10^{-6} M) stimulated morphine production (P<0.001, One Way ANOVA compared to tyramine stimulated ganglia morphine levels). Each experiment was repeated 5 times and the mean plus/minus the SEM graphed.

size particle was used to purify dopamine. Isocratic mobile phase was applied with one buffer (1 mM CH3COONH4 in 98% water and 2% Acetonitrile; Fisher Scientific). Follow rate was set at 0.5 ml/min. Concentration curve was obtained by running different concentrations of dopamine. The detection limit was 0.5 µg/ml.

CYP2D6 molecular demonstration

Isolation of total RNA

Pedal ganglia (100) were immediately processed after dissection. The ganglia were placed in 1.5 ml tubes and then washed with PBS (Invitrogen, Carlsbad, Ca). Total RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, Ca). Ganglia were homogenized in 600 µl lysis buffer. The samples were then processed following the manufacturer's instructions. In the final step, the RNA was eluted with 50 µl of RNase-free water by centrifugation for 1 min at 10,000 rpm.

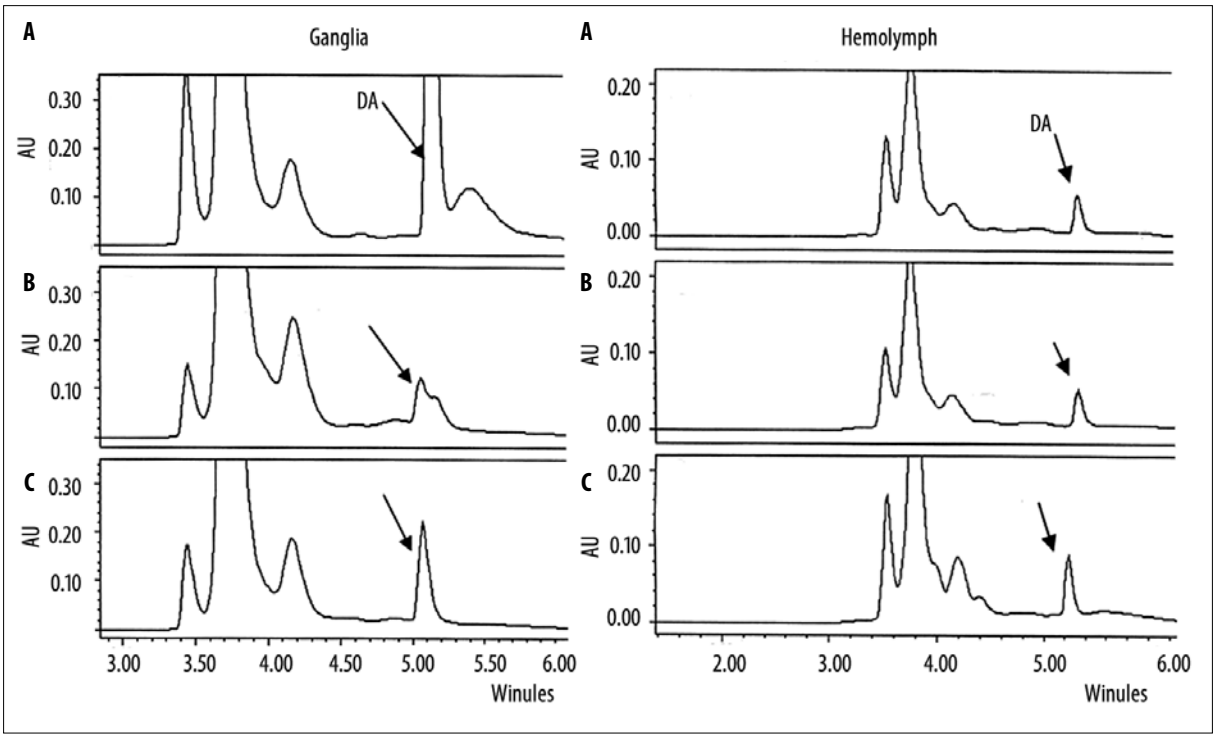


Figure 4. Representative HPLC chromatograms demonstrating ganglionic and hemolymph dopamine (DA) levels can be modulated by tyramine and quinidine exposure. **Pedal Ganglia:** (**A**) Tyramine injection (100 µg/animal under foot) 9.17±1.21 µg/g of DA. (**B**) Tyramine and quinidine injections (100 µg/animal) lower DA levels (2.57±0.32 µg/g) and (**C**). PBS injection, control DA levels 4.78±0.27 µg/g. **Hemolymph:** (**A**) PBS incubation, control DA levels (10.13±0.34 µg/ml). (**B**) DA level following tyramine and quinidine exposure to pedal ganglia (levels of Tyr 100 µg/ml, quin 10 µg/ml and DA 10.24 µg/ml). (**C**) Tyramine incubation increases DA levels (Tyramine 100 µg/ml and DA 16.47±2.28 µg/ml).

Reverse transcription-coupled polymerase chain reaction (RT-PCR)

First-strand cDNA synthesis was performed using random primers (Invitrogen, Carlsbad, CA). 1 µg of total RNA was denatured at 95°C and reverse transcribed at 40°C for 1 hr using Superscript III Rnase H-RT (Invitrogen, Carlsbad, CA). 10 µl of the RT product was added to the PCR mix containing primers for the CYP2D6 and CYP2D7 gene and Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). The forward primer sequence was 5'-GGCCAAGGGGAACCCTGAGA-3' and reverse primer was 5'-GGTCATACCCAGGGGGACGA-3'. The PCR reaction was denatured at 95°C for 5 minutes followed by 40 cycles at 95°C for 30s, 60°C for 30s, and 72°C for 1 min, and then an extension step cycle at 72°C for 10 min. PCR products were analyzed on a 2% agarose gel (SIGMA, St. Louis, MO) stained with ethidium bromide. The expected sizes of the PCR products were 282 bp for CYP2D6 and 340bp for CYP2D7, as described by [14].

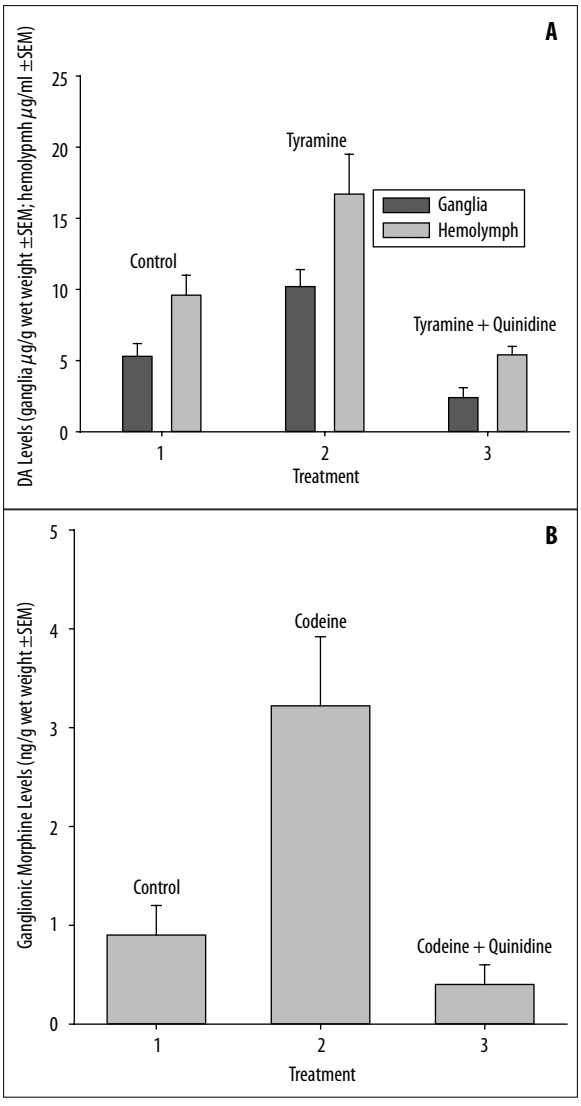
DNA sequencing

After excising the PCR product from the gel, DNA purification was performed with the Qiaquick gel extraction kit (Qiagen). The PCR product was dissolved in 35 µl H_2O and sent to Lark Technologies, Inc. (Houston, TX) for direct sequencing.

Results

M. edulis pedal ganglia were incubated *in vitro* with tyrosine or tyramine, two putative morphine precursors. This resulted in increases in ganglionic morphine levels in a concentration- and time-dependent manner (Figure 1; $P<0.01$, from 1.08±0.27ng/g ganglionic wet weight to 2.51±0.36 ng/g for tyrosine, from 0.96±0.31ng/g to 2.39±0.64 ng/g for tyramine). The increase in ganglionic morphine levels, after tyrosine and tyramine exposure, occurred gradually over the 60 min incubation period (Figure 1B). We estimate from the applied concentration of the respective precursors that approximately 5% of tyrosine or tyramine gets converted to morphine under these *in vitro* conditions. Blank runs between morphine HPLC determinations, as well as running negative tissue controls, e.g., mantle, did not show a morphine residue with HPLC coupled RIA. Analysis of the artificial seawater, concentrated marine bacteria and algae in the aquarium and various chemicals used in the protocol also demonstrated a lack of morphine. Additionally, in all experiments there is no difference between saline and non-treated ganglia.

Since published reports demonstrate CYP2D6 involvement in morphine biosynthesis we examined this process for its activity and presence. The CYP2D6 inhibitor, quinidine [14], in a concentration dependent manner, decreased ganglionic morphine levels when ganglia were exposed to tyramine as did AMPT, a tyrosine hydroxylase inhibitor, when ganglia were exposed to tyrosine (Figures 2,3). Exposure to either enzyme inhibitor alone did not significantly reduce morphine levels below the level of non-exposed ganglia (AMPT at 10^{-5} M = 0.79±0.18 ng/g wet weight ±SEM (Figure 2); quinidine at 10^{-6} M=0.73±0.21 ng/g wet weight ±SEM (Figure 3); compared to control values =0.99±0.16 ng/g wet weight ±SEM, Figure 3). However, exposure of pedal



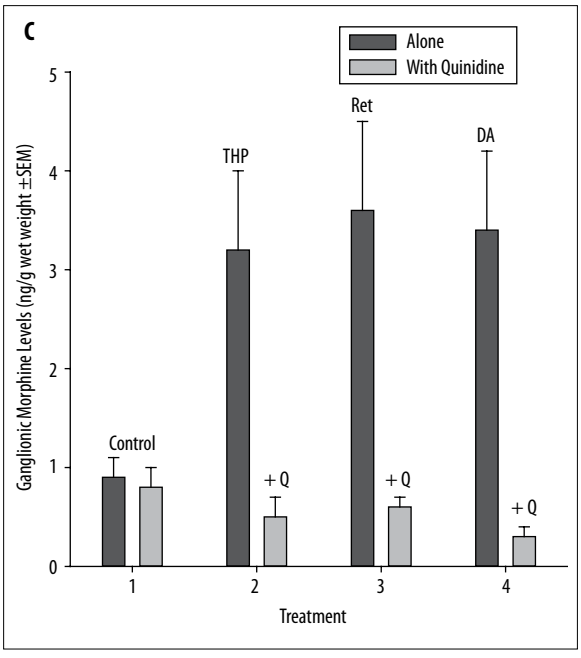


Figure 5. Modulation of ganglionic DA and morphine levels at various CYP2D6 mediated steps in the morphine biosynthetic pathway. (**A**) Quinidine (10^{-6} M), the CYP2D6 antagonist, blocks the increase in endogenous ganglionic and hemolymph DA levels caused by the exposure of the pedal ganglia to tyramine alone (P<0.001). (**B**) Quinidine blocks the increase in endogenous ganglionic morphine levels stimulated by codeine exposure (10^{-6} M)(concentrations and levels of morphine compared to just administering codeine alone (T-test, P<0.001). (**C**) Norlaudanosoline (THP, 10^{-6} M), reticuline (10^{-6} M) and DA (10^{-6} M) when combined with quinidine significantly block the increase in endogenous morphine levels they stimulate when administered alone (concentrations and levels of morphine compared to when they are administered alone; T-Test where P<0.001).

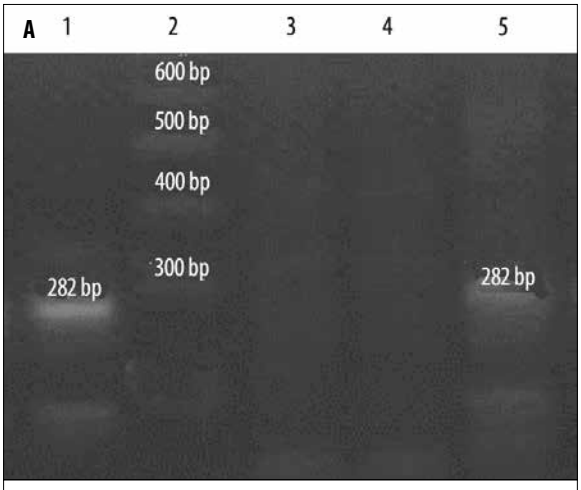
ganglia to both enzyme inhibitors did reduce ganglionic morphine levels significantly (0.23±0.16 ng/g wet weight ±SEM; P<0.01) below that of controls. These results therefore indicate compensatory effects between both pathways, here operating simultaneously.

In order to further determine the significance of CYP2D6 in ganglionic morphine biosynthesis, we examined two of the proposed mediation steps in this process. In the tyramine to dopamine step, we examined DA levels in ganglia and hemolymph before and after tyramine addition, followed by CYP2D6 inhibition by quinidine (Figures 4,5). Tyramine injection significantly increased ganglionic dopamine (4.98±0.27 μg/g to 9.17±1.21 μg/g wet weight; P<0.01) and hemolymph levels (10.13±1.24 μg/ml to 16.47±1.28 μg/ml P<0.05). The DA ganglionic and hemolymph level increases were blocked by quinidine exposure. Next, we exposed ganglia to THP, reticuline, DA and codeine, which resulted in significantly enhanced morphine levels, a phenomenon that was also significantly blocked by quinidine exposure (Figures 4,5).

Supporting this indirect pharmacological evidence for CYP2D6 in the ganglia, we demonstrate its presence on a molecular level. RT-PCR analysis amplified a 282 bp fragment, demonstrating the presence of CYP2D6 mRNA [14] (Figure 6A). Sequence analysis of this transcript fragment demonstrated a 94% similarity to human GeneBank accession number M20403 (Figure 6B).

In the *in vivo* experiments, animals were injected with either tyrosine or tyramine in their foot (Figure 7). One hour after injection, ganglia were dissected and extracted for morphine analysis. Both precursors significantly enhanced ganglionic morphine levels compared with control values (2.46±0.22 ng/g wet weight for tyrosine and 3.28±0.45 ng/g for tyramine compared to controls 1.02±0.24ng/g; P<0.005; Figure 7). Statistical significance could not be achieved with injection concentrations of 10^{-7} to 10^{-6} M, but was significant at a level of 10^{-5} M (Figure 7). Additionally, 20 animals per drug protocol were injected via the foot with either AMPT (10^{-4} M) or the CYP2D6 inhibitor, quinidine (10^{-4} M). These animals did not exhibit any change in their morphine levels even when co-administered (data not shown).

Compared to controls injected with saline, the tyrosine and tyramine animals exhibited a significant decrease in gan-



B

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Figure 6. CYP2D6 in *M. edulis* pedal ganglia. (**A**) Agarose gel showing PCR products obtained from various cDNA. Lane 1, positive control (bright band =282 bp); lane 2, 100 bp ladder (100–600 bp); lane 3, negative reagent control; lane 4, *M. edulis* mantle tissue another negative control; lane 5, Ganglia (bright band = 282 bp). (**B**) Partial CYP2D6 sequence obtained via RT-PCR demonstrates a 94% similarity to GenBank accession number M20403, **Top strand** shows position 843-1107, GenBank accession number M20403; **Bottom strand** is *M. edulis* cDNA sequence. Bold represent mismatches, no data and gaps

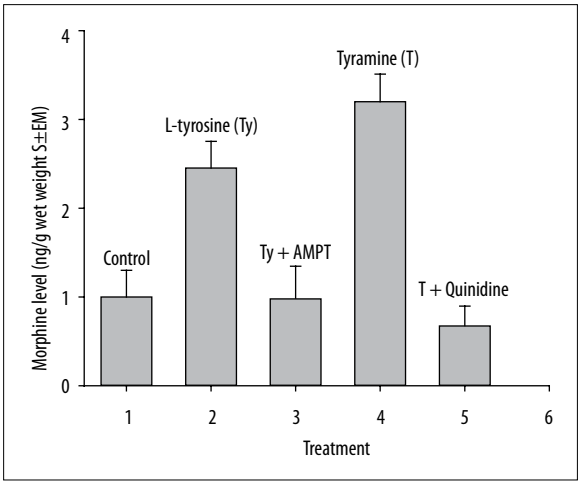


Figure 7. Tyrosine and tyramine foot injection in healthy animals increases endogenous pedal ganglionic morphine levels *in vivo*. 20 animals were injected either with the respective morphine precursors (100 µg/animal) and other animals had their pedal ganglia exposed to the tyrosine hydroxylase inhibitor, AMPT (100 µg/animal) or the CYP2D6 inhibitor, quinidine (100 µg) 15 min post injection. Compared to controls, injected with saline, the tyrosine and tyramine animals exhibited a significant increase in ganglionic morphine levels that was blocked by the respective enzyme inhibitor only when applied to the ganglia not co-injected in the foot. (ANOVA, $P<0.001$). There is no significant difference between control, control injected with AMPT, and control injected with quinidine.

glionic morphine levels when the respective enzyme inhibitors were topically applied to the pedal ganglia of intact animals after they were injected with the respective precursors in the foot (decrease of 30 and 25%, respectively; $P<0.01$). In this regard, we estimate that less than 0.5% (0.26% for tyrosine and 0.38% for tyramine.) of the injected precursors went directly to morphine biosynthesis under the *in vivo* conditions.

Discussion

The present study demonstrates that exposing pedal ganglia to the putative morphine precursors, tyrosine and tyramine [17] results in significant increases in ganglionic morphine levels, which are time- and concentration-dependent. We also demonstrate, both pharmacologically and pharmacologically and by molecular analysis, that CYP2D6 and tyrosine hydroxylase are involved in this process. Additionally, hemolymph does reflect the ganglionic treatments, suggesting that under normal conditions these chemical messengers originate via secretion from the nervous tissues. Taken together, it is apparent that *M. edulis* neural tissues have the ability to synthesize morphine from tyramine or tyrosine and CYP2D6 and tyrosine hydroxylase are involved in this process. CYP2D6 presence and role is further supported by the finding of many cytochrome P450 isoforms in *M. edulis* tissues [18].

In further support of the animal's ability to synthesize morphine, we note that morphine precursors have been identified in normal and healthy mammalian and invertebrate tissues [1,19–22], including parasites [23–27]. The first report of an intact animal producing morphine was in the parasite *Ascaris suum* [24]. The presence of endogenous morphine and its role as a chemical messenger in animals was further verified by the cloning of the mu3 opiate receptor subtype, found in human and invertebrate tissues, which only responds to opiate alkaloids (e.g., morphine), as opposed to opioid peptides, providing the means for its signaling [5,28].

The argument for a *de novo* biosynthetic pathway in healthy animals also can be supported by studies demonstrating the ability of animal enzymes to synthesize, through the same precursors, morphine in an identical stereo- and regio-specific manner to that of the poppy plant [4,21,29,30]. In this regard, we demonstrate a role for cytochrome P450 isoforms in morphine animal biosynthesis, since it can perform functions required of this synthesis, e.g., hydroxylation [31]. Supporting this hypothesis is the demonstration that CYP450 isoforms, e.g., CYP2D6, can convert codeine to morphine [14] and tyramine to dopamine [32]. The present study supports this role for the cytochrome P450 enzyme family.

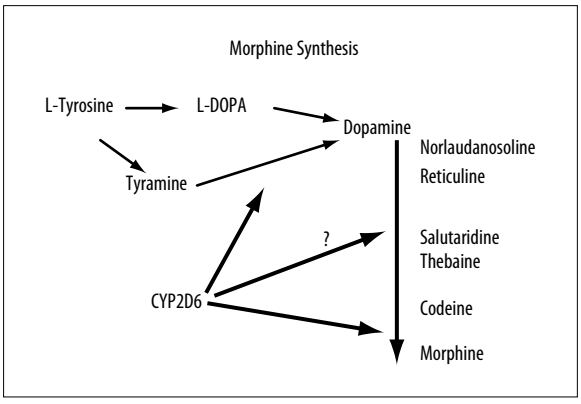


Figure 8. Morphine formation in Mytilus Pedal Ganglia. The various inhibitors and types of inhibition employed demonstrate that CYP2D6 inhibits the conversion of tyramine to dopamine as well as steps in the synthesis of morphine from dopamine and tyrosine hydroxylase (TH) is involved in the formation of morphine from tyrosine. This demonstrates that morphine can be made from two starting points. Taken together with the data found in the report, we also demonstrate that inhibiting either pathway separately results in continued morphine synthesis because the other pathway can compensate for this inhibition.

We now demonstrate quinidine inhibition of the pathway as well as the CYP2D6 role in the conversion of tyramine to dopamine, reticuline, THP, DA and codeine metabolism to morphine. Our results also support the presence of this enzyme in this invertebrate ganglion since the isolated mRNA fragment and resulting RT-PCR product, exhibits 94% sequence identity with its human counterpart [14]. These results place CYP2D6 in a pivotal position in the biosynthesis of morphine in animals (Figure 8).

Furthermore, the study revealed that morphine biosynthesis may occur in *M. edulis* pedal ganglia by way of tyrosine and/or tyramine, making it very likely that this synthesis would occur regardless of circumstances. As demonstrated, neither AMPT nor quinidine when administered alone dropped endogenous morphine levels below that of controls. Co-administration did, however, cause this to occur. This suggests that basal levels were being maintained via morphine storage, or the inhibitors did not reach the ganglia. The enzyme inhibitor data suggest that if one pathway is blocked, the overall pathway continued because the other pathway to dopamine compensated. In this regard, one must ask what is so critical about morphine biosynthesis that this process can occur from two starting points, ensuring its production.

In the animals examined for the presence of morphine as of yet, this signal molecule increases its basal levels significantly after stress or trauma [7,10,33]. In numerous reports we have identified the related receptor, designated mu 3, on nervous, immune, vascular and gut tissues where it is coupled to constitutive nitric oxide synthase stimulated nitric oxide release [7,34,35]. We have surmised that endogenous morphine, in general, is involved with down regulating various physiological activities (e.g., nervous, immune, vascular, and gut) [7,17,34–42]. Finding morphine in mammalian limbic tissues supports this view as well by linking it to emotional reward systems, previously emphasizing a role for dopamine [37,38,41–51]. Thus, given morphine's widespread ability to limit and inhibit excitation**,** this function is visibly quite important for the survival of all animals, hence its ability to be synthesized via two critical routes.

It is also important to note the linking of morphine biosynthesis to that of DA [17]. This coupling to dopaminergic processes has important biomedical significance. For example, in Parkinson's Disease after L-DOPA exposure or exercise, elevated morphine levels were found [43,52]. This link is equally important when considering animal behavior. We surmise that the DA component modulates excitatory states, including rage, whereas the morphinergic component offers calming action associated with relaxation and reward [7,34,37,42,45–47,53–56]. This association may also explain the calming effect following excitatory emotional states. Moreover, in this scenario of DA synthesis coming before that of morphine, one would predict excitation would precede the calm, which may be associated with morphine signaling. Furthermore, this coupling may also explain the fact that, within various relaxation techniques, an excitatory stress component emerges physiologically before relaxation sets in [53,54,57].

CONCLUSIONS

Taken together, we demonstrate that, in fact, animals have the ability to make morphine. CYP2D6 appears to be an important enzyme in this pathway and morphine can be made from tyramine or tyrosine, which appears to compensate during processes that may compromise morphine synthesis. We also establish a link between DA and morphine biosynthesis that promises to be the subject of future investigations given its significance in biomedicine.

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