Response to Comment on "Human White Blood Cells Synthesize Morphine: CYP2D6 Modulation"

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*J Immunol* 2006; 176:5704; doi: 10.4049/jimmunol.176.10.5704

http://www.jimmunol.org/content/176/10/5704
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ttefano et al. (1) observed that human polymorphonuclear (PMN) cells contained and biosynthesized morphine endogenously. We confirm here, by gas chromatography-tandem mass spectrometry (2, 3), the presence of morphine in human PMN cells (10.4 ± 1.6 pg/million cells) but furthermore also in mononuclear (MN) cells (8.5 ± 1.0 pg/million cells) and the erythrocyte fraction (81.3 ± 7.4 pg/ml packed volume) (morphine contamination excluded). It was also suggested (1) that morphine is biosynthesized in PMN from tyramine via conversion to dopamine by action of CYP2D6. Applications of unlabeled and [ring-13C6]tyramine (10⁻⁶ M) to PMN or MN as published (1) showed no significant increase in morphine level (<0.5%) (Fig. 1), and absolutely no labeled morphine (neither 13C₁₀- nor 13C₁₂-morphine) was detected. Furthermore, the incubation of PMN or MN cells with [ring-3,5-[3H]₂]tyramine showed no significant release of ³HOH from [ring-3,5-[3H]₂]tyramine (4), indicating that 3-hydroxylation of tyramine did not occur, and thus, dopamine was not formed by PMN or MN cells. In addition, we found that [1-13C₃,3⁻¹⁸OH]dopamine was transformed by PMN or MN to its metabolites, [1-13C₃,3⁻¹⁸OH]-3,4-dihydroxyphenylacetic acid (DOPAC) (identified as trimethylsilyl (TMS) derivative: m/z 387 [M]⁺, 16.4 ± 1.0 ng/million cells) and [1-13C₃,3⁻¹⁸OH]homovanillic acid (HVA) (as TMS derivative: m/z 329 [M]⁺, 18.8 ± 3.1 ng/million cells) (Fig. 2). Neither DOPAC nor HVA was formed in PMN or MN supplied with either unlabeled or [ring-13C6]tyramine, again showing that tyramine was not transformed to dopamine. [6-²H]Codine applied to PMN or MN was demethylated to morphine (as TMS derivative: m/z 387 [M]⁺, 16.4 ± 1.0 ng/million cells) and [1-13C₃,3⁻¹⁸OH]homovanillic acid (HVA) (as TMS derivative: m/z 329 [M]⁺, 18.8 ± 3.1 ng/million cells) (Fig. 2), showing the presence of functionally active CYP2D6 in PMN or MN (Fig. 1). Three types of experiments presented here show clearly that isolated human white blood cells (PMN and MN) do not transform tyramine by action of CYP2D6 to dopamine and fail to synthesize morphine.

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FIGURE 1. Morphine (as TMS derivative) detection and enhancement following precursor exposure. Human white blood cells (WBC) isolated from heparinized blood were incubated with unlabeled [ring-13C₆]tyramine and [3⁻²H]codeine at 10⁻⁶ M for 1 h. WBC morphine significantly increased when incubated with codeine (2600 ± 700 pg/10⁶ cells), but neither with unlabeled tyramine (10.5 ± 0.7 pg/10⁶ cells) nor with [ring-13C₆]tyramine (11 ± 3.4 pg/10⁶ cells), compared with a control value of (10.4 ± 1.6 pg/10⁶ cells). Each experiment was repeated four times, and the mean ± SEM was graphed.

FIGURE 2. Dopamine-derived acids (as TMS derivative) detection and enhancement following precursor exposure. Human WBC isolated from heparinized blood were incubated with unlabeled [ring-13C₆]tyramine and [1-13C₃,3⁻¹⁸OH]dopamine at 10⁻⁶ M for 1 h. WBC DOPAC and HVA significantly increased when incubated with labeled dopamine (DOPAC: 16,400 ± 1,000 pg/10⁶ cells, HVA: 18,800 ± 3,100 pg/10⁶ cells), but neither with unlabeled tyramine (76 ± 8.5 pg/10⁶ cells nor with [ring-13C₆]tyramine (67 ± 9.2 pg/10⁶ cells), compared with a control value of (56 ± 10.2 pg/10⁶ cells). Each experiment was repeated four times and the mean ± SEM was graphed.
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We are very pleased that our article has engendered such interest in the biologically important realm of endogenous morphine expression in eukaryotes by the group noted for its work in opiate alkaloid biosynthesis in plants. We now provide a point-by-point explication of potential confounds in the analyses of Boettcher et al. as follows:

1) Boettcher et al. corroborate, via gas chromatography-tandem mass spectrometry, that morphine is found in white blood cells (WBC) without commenting on the remarkable concurrence of cellular concentration values (~10 pg morphine/million cells) with our values validated by quadrupole-time of flight-mass spectrometry.

2) They conclude that WBC do not synthesize morphine originating from CYP2D6-catalyzed conversion of tyramine into dopamine without addressing our results, supporting a parallel pathway of morphine biosynthesis that derives from tyrosine hydroxylase-catalyzed conversion of tyrosine to 3,4-dihydroxy-L-phenylalanine.

3) They used our published incubation conditions, without success, to metabolically label the pool of cellular morphine from tyramine without addressing the requisite sensitivity of our RIA/HPLC detection system capable of quantification of fmol concentrations of morphine. The low sensitivity of their detection system is indicated by the 7-day incubation period that was required to monitor 13C isotope enrichment into 13C-labeled morphine from precursors in neuroblastoma cells (1, 2). The lack of consideration in determining detection limits in the labeling of various intracellular substrate pools in SHY5Y (3) may have also weakened their conclusions pertaining to the metabolic and biosynthetic pathway for morphine in human cells (1, 2).

4) The inability to detect significant levels of 3H2O arising from CYP2D6-catalyzed ring hydroxylation of [3H]tyramine is predictable based on the low specific activity of commercially available [3H]tyrosine or [3H]tyramine (typically 50 Ci/mmol for 3H3,3H5, ring-labeled tyrosine) and the inappropriate adaptation of an in vitro assay originally developed to monitor tyrosine hydroxylase activity (2, 4). The feasibility of the [3H]tyramine/[3H2O] release assay as an accurate and sensitive method for monitoring CYP2D6 activity is not established in vitro or in viable cell cultures. Boettcher et al. selectively present data indicating conversion of isotopically labeled dopamine to [1-13C, 3-18OH]-3,4-dihydroxyphenylacetic acid and [1-13C, 3-18OH]homovanillic acid without addressing the basic issue of whether isotopically labeled tyramine is directly converted to dopamine by CYP2D6, which is consistent with our published work (5) and that of others (6–9).

5) They demonstrate the presence of both monoamine oxidase and catechol-O-methyltransferase enzymes previously identified as critical for morphine biosynthesis (1, 2). Additionally, the authors demonstrate conversion of radiolabeled codeine to morphine in WBC, although they have not used appropriate CYP2D6 inhibitors. This is not surprising based on in vitro studies demonstrating that CYP2D6 demethylation occurs faster than ring hydroxylation, i.e., rapid conversion of codeine to morphine (8, 9). In light of the above, we do not understand why the authors conclude that WBC do not synthesize morphine.

6) In conclusion, our results demonstrating a relatively slow conversion of tyramine, via dopamine, into morphine are supported by our enzymology studies of CYP2D6 showing a 10-fold lower binding affinity of tyramine to the active site of the enzyme as compared with the 3-OH-analog (8, 9).

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