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# Fast Access of Some Grape Pigments to the Brain

SABINA PASSAMONTI,[†] URSKA VRHOVSEK,[§] ANDREJA VANZO,[†,#] AND FULVIO MATTIVI*,[§]

Dipartimento di Biochimica Biofisica e Chimica delle Macromolecole, Università di Trieste, via L. Giorgeri 1, 34127 Trieste, Italy; Istituto Agrario di San Michele all'Adige (IASMA), Centro Sperimentale, via E. Mach 2, 38010 San Michele all'Adige, Italy; and Kmetijski Institut Slovenije, Hacquetova 17, 1001 Ljubljana, Slovenia

Anthocyanins represent the main flavonoid pigments in red grape and wine, in red berries, and in many other fruits and vegetables and are widespread in the human diet. After ingestion, these complex, hydrophilic compounds quickly appear as intact molecules in the plasma. This study investigated their presence in the brain of anesthetized rats that received 8 mg/kg of body weight of a pure anthocyanin mixture extracted from *Vitis vinifera* grapes. The mixture was maintained in the stomach for 10 min. After this time, intact anthocyanins were detected by HPLC-DAD-MS not only in the plasma (176.4 ± 50.5 ng/mL, mean ± SEM) but also in the brain (192.2 ± 57.5 ng/g). These results demonstrate for the first time that grape pigments can reach the mammalian brain within minutes from their introduction into the stomach.

**KEYWORDS: Flavonoids; anthocyanins; plasma; brain; pharmacokinetics; biological availability**

## INTRODUCTION

Wine has been an element of the human diet in the Mediterranean area since millennia. Moderate wine consumption has been associated with a significantly lower incidence of dementia (*1−4*), and this has been attributed to the action of flavonoids (*5*). The strong inverse correlation between dietary intake of flavonoids and the risk of developing dementia raises the issue of whether these molecules can act directly at the level of the central nervous system. However, this hypothesis remains to be tested.

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (*6*), which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (*7*). In addition, it has been pointed out that flavonoids might act as effectors in cell signaling (*8*), with the potential to improve cell connections and neurogenesis (*9*). The ultimate implication is that the prevention of neurodegenerative diseases could be managed through diets rich in flavonoids (*10*).

Anthocyanins are the most abundant flavonoid pigments occurring in red grape and other fruits, vegetables, and wines (*11*). Following ingestion, they are quickly detected in the plasma, although at very low levels (*12−17*). To investigate whether these compounds may actually reach the brain, we administered a solution of pure anthocyanins extracted from *Vitis vinifera* to anesthetized rats via an intragastric route. First, we observed that levels of intact anthocyanins in the plasma were similar to those obtained following administration of similar solutions by the same way (*18*). Second, we demonstrate for the first time that, under these conditions, intact anthocyanins reached the brain within minutes from their introduction into the stomach.

## MATERIALS AND METHODS

**Animals.** Male Wistar rats (250−270 g) were fed standard laboratory chow (Harlan Teklad 2018) and tap water ad libitum; they were housed in temperature-controlled rooms at 22−24 °C and 50−60% relative humidity. Their care and handling were in accordance with the provisions of European Community Council Directive 86-609. They were fasted for 24 h before the experiment.

**Other Materials.** Two and a half milliliters of a 2.5% (mass/vol) solution of 2,2,2-tribromoethanol (Sigma-Aldrich, Steinheim, Germany) in ethanol/0.15 M NaCl (1:9, v/v) was used to anesthetize rats. Methanol (HPLC grade, Carlo Erba, Milano, Italy) was used for the extraction of anthocyanins from rat tissues. Formic acid (HPLC grade, Carlo Erba) was used for the HPLC analysis.

**Preparation of the Anthocyanins Mixture.** Anthocyanins were isolated from *V. vinifera* (cv. Cabernet Sauvignon) berries as previously described (*19*). The mixture prepared for feeding experiments was analyzed by HPLC (see below) and was found to contain the following anthocyanins [the percentage represents the proportion of individual peaks relative to the total chromatogram area ($\lambda$ = 520 nm)]: delphinidin 3-glucoside, 11.89%; cyanidin 3-glucoside, 1.94%; petunidin 3-glucoside, 13.83%; peonidin 3-glucoside, 9.26%; malvidin 3-glucoside, 47.18%; delphinidin 3-(6-*O*-acetyl)glucoside, 2.15%; cyanidin 3-(6-*O*-acetyl)glucoside, 0.24%; petunidin 3-(6-*O*-acetyl)glucoside, 2.21%; peonidin 3-(6-*O*-acetyl)glucoside, 1.24%; malvidin 3-(6-*O*-acetyl)glucoside, 7.49%; delphinidin 3-(6-*O*-*p*-coumaroyl)glucoside, 0.12%; cyanidin 3-(6-*O*-*p*-coumaroyl)glucoside, 0.03%; petunidin 3-(6-

* Author to whom correspondence should be addressed (telephone +39-0461-615259; fax +39-0461-615288; e-mail fulvio.mattivi@iasma.it).
[†] Università di Trieste.
[§] Istituto Agrario di San Michele all'Adige (IASMA).
[#] Kmetijski Institut Slovenije.

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**Table 1.** Limit of Detection (LOD, s/n = 3), Limit of Quantification (LOQ, s/n = 10), and Repeatability for Mass Spectrometry and Diode Array Detection of Pure Anthocyanins (∼600 ng/mL, $n = 12$)

| | | | detection by MS, single ion monitoring | | | diode array detection, 520 nm | | |
|---|---|---|---|---|---|---|---|---|
| anthocyanin | *m*/*z* | cone voltage (V) | LOD (nM) | LOQ (nM) | repeatability (CV, %) | LOD (nM) | LOQ (nM) | repeatability (CV,%) |
| malvidin 3-glucoside | 331.2 | 65 | 19.1 | 63.8 | 2.31 | 37.1 | 123.7 | 0.74 |
| petunidin 3-glucoside | 317.2 | 50 | 51.5 | 171.5 | 1.96 | 44.2 | 147.3 | 0.96 |
| delphinidin 3-glucoside | 303.2 | 50 | 84.8 | 282.7 | 5.02 | 33.9 | 113.0 | 0.85 |
| peonidin 3-glucoside | 301.2 | 50 | 16.1 | 53.5 | 2.18 | 26.8 | 89.3 | 1.03 |
| cyanidin 3-glucoside | 287.2 | 50 | 38.3 | 127.6 | 5.32 | 33.4 | 111.5 | 0.83 |

*O-p*-coumaroyl)glucoside, 0.40%; peonidin 3-(6-*O-p*-coumaroyl)glucoside, 0.17%; malvidin 3-(6-*O-p*-coumaroyl)glucoside, 1.86%.

**Standards of Anthocyanins.** Delphinidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-glucoside were purified and crystallized as chloride salts with purity of >98%, according to the procedure already reported (*19*). Cyanidin 3-glucoside chloride was obtained from Polyphenols Laboratories (Sandnes, Norway) and was certified from the producer for a purity of >97%.

**Administration of the Anthocyanin Mixture to Anesthetized Rats.** The surgical procedure for administering anthocyanins into the stomach of anesthetized rats was identical to that previously described (*18*). It was both compliant with the European Union law and approved by the University of Trieste animal experimentation authority prior to the initiation of the study. Briefly, it consisted of the dissection of the abdominal wall of the rat, the fixing of a ligature around the cardias, and the sliding of plastic tubing (connected to a syringe) into the stomach through a slit in the duodenum. After extensive rinsing, the stomach was filled with 4 mL of an acidified saline solution (10 mM HCl/0.15 M NaCl) containing 2 mg (3.8 $\mu$mol) of the pure anthocyanin mixture. After 10 min, 0.1 mL (500 IU) of sodium heparin (Clarisco, Schwarz Pharma S.p.A., Milano, Italy) was injected into the inferior cava vein. Immediately thereafter, the thorax was opened and 3−4 mL of systemic blood were obtained by cardiac puncture. The rat was euthanized by decapitation. The whole brain was quickly excised (cutting or crushing was avoided as much as possible). The brain was carefully rinsed in ice-cold phosphate buffered (pH 7.4) saline solution to remove traces of meningeal blood and weighed.

**Preparation of Plasma Extracts.** Plasma was obtained by centrifugation of blood samples, and anthocyanins were extracted with methanol as described in ref *18*. Extracts were stored at −20 °C until sent to the analytical laboratory for the further steps.

**Preparation of Brain Extracts.** The brain was directly homogenized and extracted into 9 volumes of ice-cold methanol saturated with nitrogen. Brain extracts were centrifuged at 3640*g* for 10 min at 4 °C. Supernatants were decanted in glass tubes under a stream of nitrogen and screw-capped. They were stored at 4 °C until sent to the analytical laboratory for the further steps, which were accomplished within 48 h.

**Cleanup of Tissue Extracts.** This procedure was carried out as described in detail in ref *18*. It consisted of evaporating methanol from the extracts, diluting the residue in acidified water, and extracting anthocyanins by solid-phase adsorption onto a hydrophobic matrix (Sep-Pak C18, 0.35 g, Waters, Milford, MA). Anthocyanins were eluted with methanol, evaporated to dryness, and immediately redissolved in a mixture suitable for injection into the HPLC columns, as specified below. Before injection, samples were filtered through a 0.22 $\mu$m PVDF filter (Millipore, Bedford, MA) into HPLC vials.

**Recovery of Anthocyanins from the Brain.** For determination of anthocyanins in control rats, rats were fasted for 24 h prior to brain removal. Whole brains were removed from rats ($N = 5$), immediately after decapitation. The brain was rinsed, weighed, and homogenized in 9 volumes of ice-cold methanol spiked with 2.26 $\mu$g of the anthocyanin mixture (20 $\mu$L of a 113 mg/L methanolic solution). Spiked brain extracts were further prepared as described above and stored at 4 °C until being analyzed by HPLC within the same day.

**HPLC−Diode Array Detection−Mass Spectrometry (HPLC-DAD-MS) Analysis.** Separation and analysis of anthocyanins in both brain and plasma extracts were performed on a Waters 2690 HPLC equipped with a Waters 996 diode array detector, a Micromass ZQ ESI-MS system (Micromass, Manchester, U.K.), and Empower software (Waters Corp.). Separation was performed using a column XTerra MS $C_{18}$ (Waters Corp.), 2.1 × 150 mm, 3 $\mu$m, with a guard cartridge. Samples were dissolved in 0.5 mL of a solution of methanol/$H_2O$/formic acid (50:47.5:2.5 v/v), and aliquots (40 $\mu$L) were injected into the column (maintained at 40 °C). The mobile phase consisted of 5% formic acid in $H_2O$ (A) and 5% formic acid in methanol (B), and the flow rate was 0.2 mL/min. The gradient employed started at 10% B with successive linear increases of B to 30, 40, 51.2, and 90% over 10, 7, 4, and 9 min. The column was equilibrated for 7 min prior to each injection. Absorption spectra were recorded from $\lambda = 230$ nm to $\lambda = 600$ nm, with detection at $\lambda = 520$ nm. The MS detector operated at a capillary voltage of 3000 V, an extractor voltage of 6 V, a source temperature of 105 °C, a desolvation temperature of 200 °C, a cone gas flow ($N_2$) of 30 L/h, and a desolvation gas flow ($N_2$) of 450 L/h. The outlet of the HPLC system was split (9:1) to the ESI interface of the mass analyzer. Electrospray mass spectra ranging from *m/z* 200 to 700 were taken in positive mode with a dwell time of 0.1 s. At the end of the 30 min run with mass spectra taken in the positive mode, a 1 min run in negative mode was added. The cone voltage (CV) was set in scan mode at the values of 65 V for the identification based on the aglycon peak and of 30 V for the identification based on both the fragment aglycon and molecular ion. The following single ions (*m/z*) were monitored for the quantification: 287.2 (CV = 50 V) for cyanidin derivatives, 301.2 (CV = 50 V) for peonidin derivatives, 303.2 (CV = 50 V) for delphinidin derivatives, 317.2 (CV = 50 V) for petunidin derivatives, and 331.2 (CV = 65 V) malvidin derivatives.

Each compound was identified on the basis of the following parameters: (1) retention time, (2) absorption spectra, (3) base fragment corresponding to the aglycon, and (4) molecular ion. Malvidin 3-(6-*O-p*-coumaroyl)glucoside was identified on the basis of parameters 1 and 2.

The calibration curves for standards malvidin 3-glucoside, petunidin 3-glucoside, delphinidin 3-glucoside, peonidin 3-glucoside, and cyanidin 3-glucoside were linear in the range of 140−4800 nM both for MS and for optical absorption at $\lambda = 520$ nm. Samples were quantified both by optical absorption at $\lambda = 520$ nm and by single ion monitoring in MS, with the external standard method, with the exception of brain malvidin 3-(6-*O-p*-coumaroyl)glucoside, which was quantified only by optical absorption at $\lambda = 520$ nm. The values were corrected for the appropriate recovery and expressed as equivalents of the corresponding 3-glucoside, nanograms per millliliter for the plasma and nanograms per gram for the brain. Further features of the method are listed in **Table 1**. The quantitative data reported in **Tables 2−4** were obtained by visible detection at $\lambda = 520$ nm. The MS values [obtained for all of the anthocyanins but brain malvidin 3-(6-*O-p*-coumaroyl)glucoside] were omitted, being practically identical because no matrix suppression effect was observed.

## RESULTS

Both plasma and brain extracts from 13 rats were analyzed for their anthocyanin contents by HPLC-DAD-MS. The modified analytical method allowed almost double the sensitivity of our previous method (*18*) and, in particular, improved repeatability for the quantification of the different compounds by factors 2.2−6.0 for MS and 2.9−8.0 for optical absorption at $\lambda = 520$ nm (**Table 1**).

In addition to the validation of the method for the analysis of pure standards (range of linearity, LOD, LOQ, repeatability),

**Table 2.** Recovery of Anthocyanins from Brain Extracts Spiked with 2.26 $\mu$g of an Anthocyanin Mixture[a]

| brain sample | delphinidin 3-glucoside | cyanidin 3-glucoside | petunidin 3-glucoside | peonidin 3-glucoside | malvidin 3-glucoside | delphinidin 3-(6-*O*-acetyl)-glucoside | petunidin 3-(6-*O*-acetyl)-glucoside | peonidin 3-(6-*O*-acetyl)-glucoside | malvidin 3-(6-*O*-acetyl)-glucoside | petunidin 3-(6-*O*-*p*-coumaroyl)-glucoside | malvidin 3-(6-*O*-*p*-coumaroyl)-glucoside | total antho-cyanins |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 22.54 | 21.89 | 23.64 | 25.38 | 28.87 | 17.61 | 25.27 | 24.72 | 30.06 | 27.25 | 23.88 | 26.58 |
| 2 | 19.78 | 22.92 | 22.70 | 24.86 | 29.58 | 23.42 | 24.55 | 23.71 | 30.18 | 18.01 | 25.10 | 26.52 |
| 3 | 27.84 | 24.80 | 25.44 | 28.21 | 36.22 | 28.92 | 30.89 | 34.70 | 40.55 | 28.81 | 38.36 | 32.70 |
| 4 | 23.07 | 24.94 | 25.37 | 25.08 | 33.35 | 25.62 | 31.40 | 32.85 | 36.44 | 33.49 | 29.95 | 30.03 |
| 5 | 17.55 | 23.82 | 18.12 | 25.70 | 29.77 | 21.00 | 25.39 | 29.52 | 34.13 | 21.90 | 32.27 | 26.24 |
| mean | 22.16 | 23.67 | 23.06 | 25.84 | 31.56 | 23.32 | 27.50 | 29.10 | 34.27 | 25.89 | 29.91 | 28.41 |
| SD | 3.88 | 1.29 | 3.00 | 1.36 | 3.13 | 4.32 | 3.35 | 4.84 | 4.43 | 6.05 | 5.84 | 2.86 |
| CV | 17.51 | 5.45 | 12.99 | 5.26 | 9.92 | 18.53 | 12.17 | 16.65 | 12.94 | 23.36 | 19.52 | 10.07 |

[a] Results are evaluated by HPLC-DAD at 520 nm and expressed in percentage. SD is the standard deviation and CV the coefficient of variation. $N = 5$.

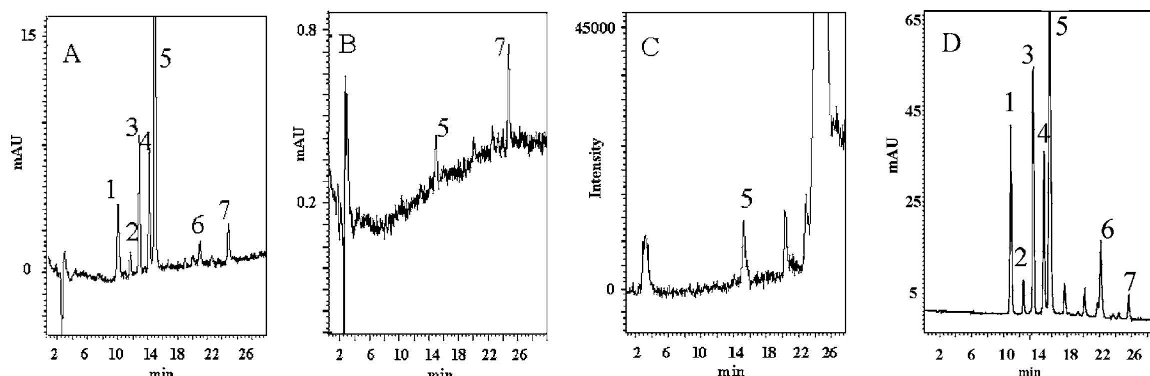


**Figure 1.** Chromatograms of either the plasma (panel **A**, visible trace at 520 nm) or the brain extract (panel **B**, visible trace at 520 nm, and panel **C**, MS trace in SIM mode $m/z$ 331.2) of rat 7. Compounds are numbered as in **Table 3**. Panel **D** shows the visible trace at 520 nm of the chromatogram of the anthocyanins mixture used for the experiment. Peak 5 is out-of-scale in panels **A** and **D**.

the accuracy of the data was assured by the analysis of blank samples and by the evaluation of the recovery from samples spiked with the anthocyanins mixture.

Preliminary tests, carried out to examine the occurrence of anthocyanins in either plasma or brain extracts (blank samples), have indicated that both matrices are anthocyanin-free. The recovery of spiked anthocyanins from brain extracts (28.4 ± 3%, **Table 2**) was lower than that from whole blood [49 ± 7% (*18*)]. The low variability between samples (**Table 2**) enabled the use of the estimated average recovery for the correction of quantitative data.

There was a negative correlation between the amount recovered and the number of free hydroxyls in the phenolic structure (i.e., malvidin > petunidin > delphinidin derivatives; free glucosides and *p*-coumaric esters < acetyl esters). This suggests that the strong interaction with the matrix is mediated by the presence of free phenolic hydroxyls, as is the case for the interaction of polyphenols with proteins and metals (*20*).

**Figure 1** shows the visible trace at 520 nm of the chromatogram of a plasma sample (panel **A**) or a brain extract (panel **B**) of rat 7. Panel **C** shows the MS trace in SIM mode at $m/z$ 331.2 of the same brain extract. For brain samples, the MS signal showed a better signal-to-noise (s/n) ratio than the visible signal for the detection of malvidin 3-glucoside (peak 5), whereas the *p*-coumaric ester (peak 7) was not detected, presumably due to the presence of colorless coeluting compounds. The visible trace at 520 nm of the chromatogram of the anthocyanins mixture used for the experiment is reported in panel **D**.

The anthocyanin content of rat plasma extracts is shown in **Table 3**. Anthocyanins were detected in the plasma of all 13 subjects. Between two and seven components of the mixture were detected in all samples. The total anthocyanin concentration in the plasma was 176.4 ± 50.5 (mean ± SEM) ng/mL (range, 30−656.4; $n = 13$). Malvidin 3-glucoside appeared as the most abundant component of the mixture administered, with an average content of 108.7 ± 29.7 ng/mL and a range of 23.4−379.8 ng/mL. The latter average value corresponds to a concentration of 0.20 $\mu$M, a value that is quite similar to that we obtained in a previous investigation [0.24 $\mu$M (*18*)].

**Table 3.** Concentration of Anthocyanins in the Plasma of 13 Rats That Received a Solution of Grape Anthocyanins Intragastrically for 10 min[a]

| rat | plasma anthocyanins (ng/mL) | | | | | | | | *n* |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | total | |
| 1 | nd | nd | 26.8 | 20.5 | 100.4 | nd | 26.3 | 174.1 | 4 |
| 2 | nd | nd | 40.8 | 36.9 | 194.4 | 19.4 | 23.6 | 315.0 | 5 |
| 3 | 28.8 | 13.5 | 61.5 | 38.9 | 244.9 | nd | nd | 387.6 | 5 |
| 4 | nd | nd | nd | nd | 42.0 | nd | 24.3 | 66.3 | 2 |
| 5 | nd | nd | 30.2 | 19.7 | 99.3 | nd | 26.2 | 175.5 | 4 |
| 6 | nd | nd | nd | nd | 45.2 | nd | 34.1 | 79.3 | 2 |
| 7 | 48.3 | 19.0 | 99.3 | 61.7 | 379.8 | 24.2 | 24.2 | 656.4 | 7 |
| 8 | nd | nd | nd | nd | 33.7 | nd | 22.2 | 55.9 | 2 |
| 9 | nd | nd | 9.7 | 30.4 | 140.4 | 13.4 | nd | 193.9 | 4 |
| 10 | nd | nd | nd | 6.6 | 23.4 | nd | nd | 30.0 | 2 |
| 11 | nd | nd | 7.0 | 6.3 | 33.1 | nd | nd | 46.4 | 3 |
| 12 | nd | nd | 6.4 | 10.2 | 43.0 | nd | nd | 59.7 | 3 |
| 13 | nd | nd | 8.3 | 11.2 | 33.0 | nd | nd | 52.6 | 3 |

[a] Compounds: **1**, delphinidin 3-glucoside; **2**, cyanidin 3-glucoside; **3**, petunidin 3-glucoside; **4**, peonidin 3-glucoside; **5**, malvidin 3-glucoside; **6**, malvidin 3-(6-*O*-acetyl)glucoside; **7**, malvidin 3-(6-*O*-*p*-coumaroyl)glucoside. *n* is the number of compounds detected; nd indicates not detected (below the limit of detection, see **Table 1**).

The anthocyanin content of rat brain extracts is shown in **Table 4**. Anthocyanins were detected in nine subjects, with an average concentration of 192.2 ± 57.5 ng/g (range, 0−567.2; $n = 13$). Malvidin 3-glucoside and its *p*-coumarate ester, which amounted to 47.2 and 1.9%, respectively, of the administered

**Table 4.** Concentration of Anthocyanins in the Brain of 13 Rats That Received a Solution of Grape Anthocyanins Intragastrically for 10 min[a]

| | brain anthocyanin (ng/g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| rat | **1** | **2** | **3** | **4** | **5** | **6** | **7** | total | *n* |
| 1 | nd | nd | nd | nd | nd | nd | nd | nd | 0 |
| 2 | nd | nd | nd | nd | nd | nd | nd | nd | 0 |
| 3 | nd | nd | nd | nd | 253.5 | nd | nd | 253.5 | 1 |
| 4 | nd | nd | nd | nd | nd | nd | 303.4 | 303.4 | 1 |
| 5 | nd | nd | nd | nd | 229.7 | nd | 337.5 | 567.2 | 2 |
| 6 | nd | nd | nd | nd | nd | nd | 356.6 | 356.6 | 1 |
| 7 | nd | nd | nd | nd | 198.0 | nd | 322.8 | 520.8 | 2 |
| 8 | nd | nd | nd | nd | nd | nd | 313.4 | 313.4 | 1 |
| 9 | nd | nd | nd | nd | 19.0 | nd | nd | 19.0 | 1 |
| 10 | nd | nd | nd | nd | 17.4 | nd | nd | 17.4 | 1 |
| 11 | nd | nd | nd | nd | nd | nd | nd | nd | 0 |
| 12 | nd | nd | nd | nd | nd | nd | nd | nd | 0 |
| 13 | nd | 11.3 | 34.7 | 36.8 | 65.0 | nd | nd | 147.7 | 4 |

[a] Compounds are numbered as in **Table 3**.

dose, were the compounds most frequently found in both tissues. The concentration of malvidin 3-glucoside appeared to be higher in the plasma (average level, 108.7 ± 29.7 ng/mL; range, 23.4–379.8 ng/mL) than in the brain (average level, 60.2 ± 27.0 ng/g; range, 0–235.5 ng/g), although the difference was not significant (*t* test, $P = 0.22$). In contrast, the concentration of malvidin 3-(6-*O*-*p*-coumaroyl)glucoside was significantly higher in the brain (125.7 ± 46 ng/g; range, 0–356.6 ng/g) than in the plasma (13.9 ± 3.8 ng/mL; range, 0–34.1 ng/mL) (*t* test, $P = 0.02$).

In individual rats, no correlation was found between the anthocyanin concentrations in the plasma and in the brain.

## DISCUSSION

The concentration of extractable anthocyanins in red grape has been estimated to vary in the range of 382–1830 mg/kg according to the variety, the lowest average value being found in the Primitivo and the highest in the Teroldego variety (*21*). An amount of 2 mg of anthocyanins could therefore have been introduced by consuming 1.09–5.24 g of grape, according to the variety. For the Cabernet Sauvignon grape, which with an average concentration of extractable anthocyanins of 871 mg/kg is close to the mean of the 25 varieties considered in the cited survey, the amount used for the experiment corresponds to the consumption of ~2.3 g of grape for each rat.

Translated on a human scale, it is a relatively high amount, corresponding to 597 g of Cabernet Sauvignon grape (or 284 g of Teroldego grape) for a person of 65 kg. The anthocyanins used in our experiments were also more readily available as they were given as an acidified saline solution and not in the whole fruit. This choice allowed us to verify the time required for the absorption in plasma and translocation in the brain, not taking into account the time required for the extraction from the fruit. In conclusion, the amount of anthocyanins administered in the present trials to each animal corresponds to a concentration attainable with a relatively large dosing of red grape. It differs in the time of contact from what can be expected in the case of daily consumption of food, whereas it could be closer both for the amount given (8 mg/kg of body weight) and for the time of contact to the consumption of dietary supplements.

In previous investigations we observed (*18*) the rapid appearance of malvidin 3-glucoside in the plasma of anesthetized rats only 6 min after the introduction into the stomach of the anthocyanin mixture. In the present work we obtained similar results, and by adopting a HPLC-MS method with improved sensitivity, we also detected a complex distribution pattern of grape anthocyanins in the plasma.

All dietary anthocyanins are glycosylated and appear as such in plasma after oral administration (*22*). The fact that they are found intact in the brain suggests that (a) deglycosylation does not take place at the blood–brain interface and that (b) similar molecular mechanisms, specifically interacting with glycosylated anthocyanins and facilitating their transcellular transport, might be involved in their passage from the gastric cavity into the blood and from the blood into the brain. It cannot, however, be ruled out from the present study that the aglycons or other metabolites can attain the brain.

Our method for extracting plasma anthocyanins recovers 89.51% of cyanidin 3-glucoside spiked in rat plasma, with a coefficient of variation of 4.76% (*18*). Thus, the wide range of anthocyanin concentration observed in the plasma of individual rats following intragastric administration of anthocyanin mixtures [also noted in humans (*13*, *23*, *24*)] appears to reflect true interindividual differences of anthocyanin absorption and metabolism rather than methodological artifacts.

Anthocyanin concentration in plasma results from a balance between absorption and removal rates. Both parameters are complex, because absorption can depend on gastrointestinal motility, blood flow, and the activity of membrane carriers. The removal rate of anthocyanins from the plasma depends on their uptake and metabolism in peripheral tissues, including excretion into bile and/or urine, and on the conversion of the intact, chromophoric flavonoid skeleton into a colorless species, due to spontaneous hydration of the C ring, followed by its conversion to a chalcone. Considering that the latter event is known to occur rapidly at pH 2–6 (*11*), it was surprising to detect some anthocyanin in plasma after the process of diffusion through the gastrointestinal barrier. The compounds detected under our conditions were exclusively of the unmodified form, which is converted into the flavylium form upon acidification. The occurrence of anthocyanins in plasma could be related to their binding to proteins, which ensures their chemical stability. Thus, the variable levels of plasma anthocyanins observed among individuals may reflect their different contents of endogenous and exogenous competitors for the protein-binding sites. The rate of anthocyanin breakdown in plasma is also influenced by a multitude of active redox compounds (*25*, *26*). The mean concentration of anthocyanins found in plasma, although low, seems to be adequate for deploying an antioxidant effect (*15*, *27*) that could be boosted in the presence of other flavonoids, such as catechin (*28*).

The rapid gastric absorption of anthocyanins implies that their appearance in plasma should normally precede that of other meal constituents, the absorption of which occurs downstream in the intestine. The early rise in anthocyanin plasma concentration that we observed and that is well documented in rats (*12*) should induce an early increase in the plasmatic antioxidant capacity (*15*), protecting the liver and other peripheral tissues from postprandial oxidative stress (*29*).

However, the real novelty of this work is the detection of significant amounts of anthocyanins in the brain after a very short exposure of the stomach to an anthocyanin mixture. The average amounts of malvidin 3-glucoside and of its *p*-coumarate ester found in the brain were similar or nearly 10-fold higher, respectively, than in the plasma. The rapid occurrence of anthocyanins in the brain prompted us not to adopt a perfusion step for the wash-out of brain blood, during which the brain would have suffered from anoxia, with unpredictable effects on the fate of these labile compounds.

The detection of anthocyanins in the brain was unexpected because this organ is thought to be impermeable to >98% of small, polar molecules occurring in the blood (*30*), due to the presence of the blood−brain barrier. Although a moderate penetration of anthocyanins in the brain was observed in an in vitro model of the blood−brain barrier, this occurred slowly, requiring hours rather than minutes (*31*). A possible explanation for our data could be that anthocyanin diffusion from the blood vessels into the brain occurred as a result of the disruption of the blood−brain barrier induced by the anesthesia and the surgical manipulation of the animal. However, there is ample evidence that anesthesia per se does not disrupt the blood−brain barrier (*32*), unless other treatments are added to anesthesia, such as infusions of osmotic solutions (*33*) or hypothermic (*34*) or hyperthermic saline solutions (*35*) or changes in cerebrospinal fluid pH (*36*), just to list a few of them. Furthermore, it should be remembered that anthocyanins have rather been shown to protect the blood−brain barrier against permeabilization (*37*, *38*).

A very recent paper, appearing during the evaluation process of the present paper (*39*), reported the detection of various cyanidin glycosides in the brain and some other tissues of the rat fed a diet including a blackberry extract. Whereas the data reported by Talavera point to the presence of these pigments in the time window between meals, and thus represent a steady-state nutritional condition, our data point to the quite rapid access of some anthocyanins into the brain, which takes place within a few minutes of their administration into the stomach. We registered an anthocyanin content in the brain 3 orders of magnitude higher than that found by Talavera, which can be justified in light of the different timing of the experiments. Taken together, the data obtained by Talavera and ourselves confirm the capacity of dietary anthocyanins from grape and berries to reach the brain and suggest that the presence of these flavonoids in the brain might take the aspect of large oscillations, depending on the incidence of these polyphenols in meals and on the time frame of the measurement.

The site of anthocyanin accumulation in the brain is not known. One possibility could be the choroidal epithelium, the tissue that controls exchanges between the cerebrospinal fluid and the blood compartment and where several membrane transporters and drug-metabolizing enzymes are expressed (*40*).

Irrespective of the route of access, the regular exposure of the brain to anthocyanins by a moderate intake of red fruits or wine might protect neurons, as also observed in rats (*41*), by combining scavenging of reactive oxygen species (*27*) with metal chelation (*42*). Both of these actions have gained attention as possible therapeutic strategies against neurodegeneration (*43*). Moreover, anthocyanins could have a direct action in cell signaling, affecting apoptosis (*44*), cell connections, and cell plasticity (*9*). This would imply specific binding of selected flavonoids by receptors and enzymes involved in cell signaling (*8*). The presence of a larger pool of flavonoid-binding sites in the brain relative to the plasma could explain our finding of some of these pigments in the brain at equilibrium.

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