Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2256–2260, April 1987
Biochemistry

Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor

(diazepam/metalloporphyrins/hemin/harderian gland)

AJAY VERMA, JEFFREY S. NYE, AND SOLOMON H. SNYDER*

Departments of Neuroscience, Pharmacology and Molecular Sciences, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Contributed by Solomon H. Snyder, December 30, 1986

ABSTRACT **"Peripheral-type" benzodiazepine receptors are localized to the outer mitochondrial membrane. We have identified potent competitive inhibitors of these receptors and purified them from human blood and from several rat organs. TLC analysis of the purified inhibitor from erythrocytes displays a single peak of inhibitory activity with an absorbance spectrum identical to hemin. All of the inhibitory activity in extracts of several tissues can be accounted for by their porphyrin and metalloporphyrin content. Pure hemin and protoporphyrin IX competitively inhibit mitochondrial benzodiazepine binding with K_i values of 41 and 15 nM, respectively, and are less active by a factor of 1000 at central-type benzodiazepine receptors. Thus, porphyrins appear to be endogenous ligands for mitochondrial benzodiazepine receptors.**

The anxiolytic effects of benzodiazepines are mediated by a "central" benzodiazepine receptor, located primarily in the brain. However, many benzodiazepines bind with high affinity to a "peripheral-type" receptor found in numerous organs whose drug specificity differs from the central-type receptor (1). The characteristic localization of this site in tissues, such as the zona glomerulosa of the adrenal gland, the testosterone-forming Leydig cells of the testes, the olfactory nerves of the brain, and the distal tubules and collecting ducts of the kidney, probably reflects a unique functional role for the receptor (1). Drugs selective for peripheral-type benzodiazepine receptors produce a pleiotropic spectrum of pharmacologic actions, including stimulation or inhibition of cell proliferation (2–4), alteration of immune function (5, 6), alteration in cardiac action potentials (7–9), effects on convulsive threshold (10–12), and alteration of protooncogene expression (13). Our subcellular localization studies indicate that the receptor is selectively associated with the outer membrane of mitochondria; hence it may be properly referred to as the "mitochondrial benzodiazepine receptor" (14, 15).

To gain insight into the physiological role of the mitochondrial benzodiazepine receptor, several laboratories have examined tissue extracts for a possible endogenous ligand. High (16) and low (16–20) molecular weight inhibitory activities have been reported in blood (17, 18), urine (17–20), and various organ extracts (16). The chemical identity has not been established for any of these inhibitory activities. In the present study we demonstrate that the low molecular weight substances in extracts of numerous tissues that compete for mitochondrial benzodiazepine receptor binding are porphyrins and that porphyrins have nanomolar affinity for the receptor.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Materials. Porphyrins were obtained from Porphyrin Products (Logan, UT) and their purity was monitored by HPLC. All other materials were provided by standard sources. Benzodiazepines {7-chloro-1,3-dihydro-1-methyl-5-(*p*-chlorophenyl)-2*H*-1,4-benzodiazepine-2-one (Ro 5-4864); ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (Ro 15-1788)} were a kind gift of Hoffman–La Roche, and 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195) was a gift of G. Le Fur.

Tissue Extractions. Packed human erythrocytes were washed twice in isotonic phosphate-buffered saline (PBS) and lysed using 3 vol of 5 mM sodium phosphate buffer. The lysate was centrifuged at 10,000 × *g* and the resulting pellet was discarded. The supernatant was diluted with 3 vol of MeOH/1 M HCl, 95:5 (vol/vol), adjusted to pH of 4.0, and extracted three times with 1000 ml of diethyl ether. The ether phase was subsequently washed twice with H_2O. At each step of the extraction small aliquots of each fraction were dried in a Speed Vac (Savant) to determine the mass/volume, reconstituted in 50 mM Tris·HCl, and assessed for potency in receptor binding assays.

Organ extracts were prepared by using an alternative procedure. Male Sprague–Dawley rats were perfused with PBS by way of transcardiac puncture under deep barbiturate anesthesia. Selected organs were dissected from surrounding fat or capsules. Organs were then minced, homogenized in 10 vol (wt/vol) of MeOH/1 M HCl, 95:5 (vol/vol), using a Polytron (Brinkmann) at speed 8 for 30 sec, and centrifuged at 10,000 × *g* for 10 min. Supernatants were collected and the pellets were extracted once again in MeOH/1 M HCl, 95:5 (vol/vol). The combined supernatants were diluted 1:5 with distilled H_2O and passed over octadecylsilane Sep-Pak columns (Waters) that were preactivated with MeOH and H_2O. The Sep-Paks were washed once with 20 ml of H_2O and the extracts were subsequently eluted using 2 ml of MeOH/1 M HCl, 95:5 (vol/vol).

Receptor Binding. Kidneys from rats perfused with PBS were dissected free of fat and surrounding capsule. Renal mitochondria were prepared as described (21). Frozen guinea pig brains (Pel-Freez) were homogenized in 50 mM Tris·HCl (pH 7.7) using a Polytron at speed 8 for 30 sec. The homogenates were centrifuged at 20,000 × *g* and the resulting pellet was washed twice in 50 mM Tris·HCl. The final pellet was resuspended in 200 vol (original wet weight) of Tris buffer.

Abbreviations: PK11195, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide; Ro 5-4864, 7-chloro-1,3-dihydro-1-methyl-5-(*p*-chlorophenyl)-2*H*-1,4-benzodiazepine-2-one; Ro 15-1788, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate.

*To whom reprint requests should be addressed.

Mitochondrial (peripheral-type) benzodiazepine receptors were assayed using [^{3}H]PK11195 [81.0 Ci/mmol (1 Ci = 37 GBq), New England Nuclear] and [^{3}H]Ro 5-4864 (79.0 Ci/mmol), the selective ligands for this receptor (1). Central-type benzodiazepine receptors were assayed using the selective ligand [^{3}H]Ro 15-1788 (22). Rat kidney mitochondria (0.05 mg of protein per ml) or guinea pig brain homogenates (0.2–0.3 mg of protein per ml) were incubated with 1 nM concentrations of ^{3}H-labeled radioligand in the presence or absence of inhibitor in a total volume of 200 μl. Nonspecific binding was determined by adding 10 μM unlabeled PK11195, Ro 5-4864, or Ro 15-1788 to the assay for the respective ^{3}H-labeled radioligand being measured.

Chromatography and Spectrophotometry. Blood and organ extracts were spotted onto silica gel TLC plates (Merck) that had been developed once in MeOH. The plates were developed either in 10:3 2,6-lutidine/H_2O in a tank saturated with 30% NH_4OH vapors (23) or in MeOH/1 M HCl, 95:5 (vol/vol).

Reverse-phase HPLC analysis (24) of extracts employed a 10-μm octadecylsilane radial compression column (Waters) equilibrated with solvent A: 45% (vol/vol) 0.1 M NaH_2PO_4 (adjusted to pH 3.5 with H_3PO_4)/55% methanol. The column was eluted at 2 ml/min with a linear gradient over 10 min from 0% to 95% solvent B, methanol. Absorbance (400 nm) was monitored to determine hemin, and fluorescence (excitation, 320 nm; emission, 520 nm) was monitored for the remaining porphyrins. Some of the porphyrins observed in the Sep-Pak eluates may have been formed during the extraction and purification procedures.

Absorbance spectra of extracts and porphyrins were obtained using a Gilford response spectrophotometer. Samples were prepared in 0.75 M pyridine/1 M KOH.

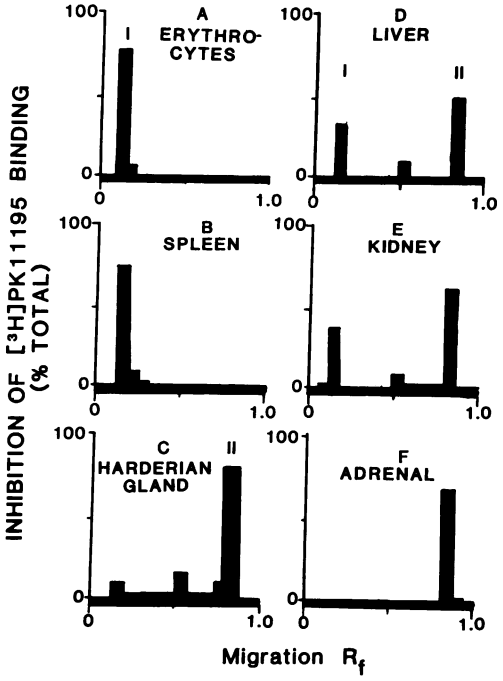

FIG. 1. TLC profiles of [^{3}H]PK11195 binding inhibitory activity extracted from several tissues. Inhibitory activity was extracted from human blood and several rat organs, spotted on silica gel TLC plates, and developed in lutidine/H_2O (10:3). The silica gel was scraped off the plates in 1-cm bands, eluted with diethyl ether, dried, and assayed for inhibition of [^{3}H]PK11195 binding to rat kidney mitochondria. (*A*) Erythrocyte ether extract. (*B*) Spleen. (*C*) Harderian gland. (*D*) Liver. (*E*) Kidney. (*F*) Adrenal gland. Two different inhibitory peaks (I and II) are distinguished by their R_f values.

RESULTS

Schoemaker *et al.* (25) reported that benzodiazepine binding of the kidney was augmented by perfusion, suggesting that blood might contain an endogenous ligand for the receptor. Erythrocyte lysates demonstrate substantial inhibition of [^{3}H]Ro 5-4864 binding to rat kidney mitochondria (Table 1). All of the activity of the lysate can be accounted for by the soluble supernatant fraction and is readily extracted into acidic methanol. Extraction with ether results in an extremely high concentration in the washed ether phase, providing almost a 300-fold purification from the initial erythrocyte lysate with a 43% recovery of activity.

TLC analysis of the ether phase utilizing two separate solvent systems (lutidine/H_2O, 10:3, methanol/1 M HCl, 95:5) reveals a single band of inhibitory activity that corresponds to the location of a visible greenish-brown spot (Fig. 1). Since all of the activity of the ether phase can be accounted for by the peak TLC fraction, the TLC procedure does not appear to afford any additional purification. The absorbance spectrum of the washed ether phase and the TLC eluate are identical to that of hemin (Fig. 2*A*). All three of these spectra demonstrate the three characteristic absorbance maxima of hemin at 399, 576, and 598 nm. Employing the extinction coefficients for hemin at these wavelengths, we calculate that at least 97% of the mass of the washed ether phase extract is hemin. Accordingly, the purification procedure provides homogenous hemin.

The ether phase extract is about 1000-fold more potent in inhibiting mitochondrial benzodiazepine receptor binding than the central-type receptor in the guinea pig brain (Fig. 3), which contains equal levels of these two receptor types (26). Authentic hemin distinguishes between the two receptors equally well, with a K_i for the mitochondrial receptor of 41 nM. Calculating from the known porphyrin content of the ether phase extract, we can account for all of the inhibition of mitochondrial benzodiazepine binding by the hemin content of the extract.

Table 1. Purification of mitochondrial benzodiazepine receptor inhibitory activity from blood

Step	[^{3}H]Ro 5-4864 inhibitory potency (IC_{50}), μg/ml	Dry mass, mg	Total activity, units	Activity/mass, units/mg	Purification, fold	Recovery, %
Erythrocyte lysate	11.9	11,375	957,492	84	1	100
Supernatant	8.1	7,500	922,509	123	1.5	96
MeOH extract	8.1	7,420	912,669	123	1.5	95
Aqueous phase	10.9	5,425	495,887	91	1.1	52
H_2O wash	4.7	424	90,106	213	3	9
Ether phase	0.042	17	411,905	23,810	283	43

Packed human erythrocytes were lysed and extracted. Each fraction was assayed for potency at inhibiting 1 nM [^{3}H]Ro 5-4864 binding to rat kidney mitochondria. One unit of activity is defined as the quantity of extract producing 50% inhibition in standard binding assays using [^{3}H]Ro 5-4864. Values shown are similar to those obtained using [^{3}H]PK11195. Data presented are representative results from two separate purifications.

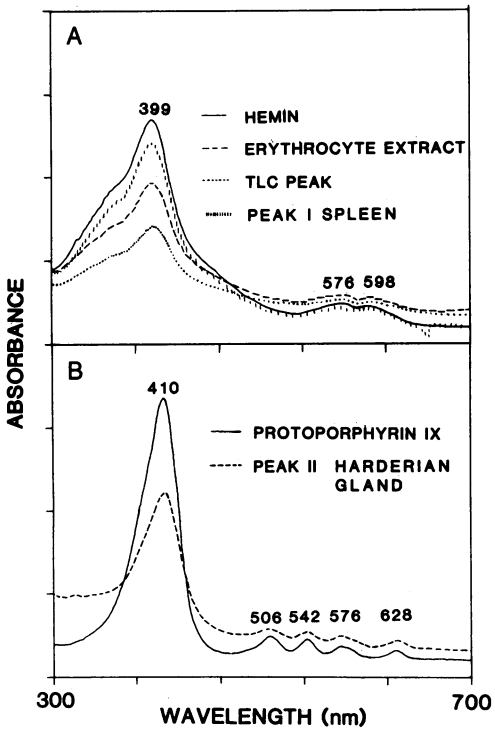

FIG. 2. Absorbance spectra of purified inhibitory extracts and porphyrins. Dried extracts were resuspended in 0.75 M pyridine in 1 M KOH for spectrophotometry. (*A*) Comparison of hemin, erythrocyte, and spleen extracts. Spectra of crystalline hemin were compared to ether extracts of human erythrocytes and samples eluted from TLC scrapings of erythrocyte and spleen (peak I) extracts (see Fig. 1). All four spectra have λ_{max} at 399 nm, with local maxima of 576 and 598 nm. (*B*) Comparison of protoporphyrin IX and harderian gland extracts. Spectra of crystalline protoporphyrin were compared to samples eluted from TLC scrapings of harderian gland extracts, peak II (see Fig. 1). Both spectra have λ_{max} at 410 nm, with local maxima at 506, 542, 576, and 628 nm.

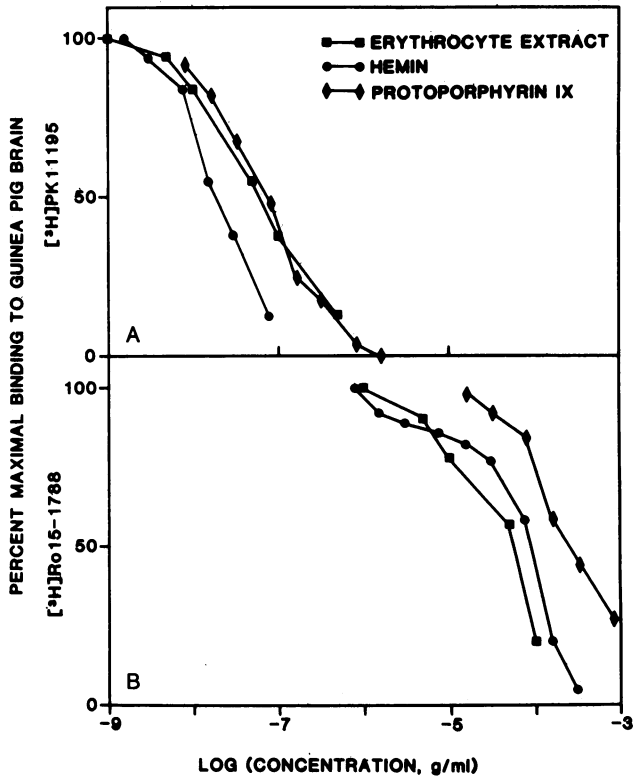

FIG. 3. (*A*) Inhibition of [^{3}H]PK11195 binding to guinea pig brain by erythrocyte ether extract, hemin, and protoporphyrin IX. Guinea pig brain membranes were prepared and assayed for mitochondrial benzodiazepine receptor binding in the presence of varying concentrations of the erythrocyte ether extract, hemin, or protoporphyrin IX. The data shown are representative of three separate experiments performed in triplicate. (*B*) Inhibition of [^{3}H]Ro 15-1788 binding to guinea pig brain by erythrocyte ether extract, hemin, and protoporphyrin IX. Central-type benzodiazepine binding was measured in the presence of varying concentrations of erythrocyte ether extract, hemin, and protoporphyrin IX. The data are representative of three experiments performed in triplicate.

We examined various peripheral tissue extracts for inhibition of mitochondrial benzodiazepine binding (Table 2; Fig. 1). Acidified methanol extracts of spleen, harderian gland, kidney, liver, adrenal gland, and brain contain substantial inhibitory activity, which was concentrated and purified by means of adsorption and elution from C_{18} Sep-Paks. The spleen extract displays the highest activity, followed by the harderian gland, kidney, liver, adrenal gland, and brain in descending order. The Sep-Pak eluate accounts for virtually all of the activity of the initial extracts. TLC analysis reveals two major peaks of inhibitory activity for [^{3}H]PK11195 binding in all of the tissues. In the spleen, this peak (peak I) migrates identically to hemin, as with erythrocyte extracts, whereas in the other tissues the peak (peak II) migrates in the same position as the dicarboxylic porphyrins, protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX, and hematoporphyrin IX. The absorbance spectra of the eluted TLC peak activity of the spleen extract (peak I) (Fig. 2*A*) are identical to that of hemin, whereas spectra of the TLC inhibitory peak in harderian gland extracts (peak II) are identical to protoporphyrin IX (Fig. 2*B*). Rats were saline perfused to remove blood from organs, but residual erythrocytes may nonetheless account for the hemin in the spleen.

Since a variety of porphyrins occur in organs, we evaluated the influence of various porphyrins and related compounds on mitochondrial receptors (Table 3). The most potent agents, with K_i values of 15–41 nM, are protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX, and hemin. Other porphyrins examined are <3% as potent as protoporphyrin IX, whose K_i is 15 nM. Scatchard analysis indicates a competitive inhibition of mitochondrial benzodiazepine binding by porphyrins.

We separated the porphyrin content of perfused rat organs by HPLC, monitoring the porphyrin content by fluorescence and absorbance characteristics (Table 4). In the spleen, hemin is the principal porphyrin with substantial amounts of mesoporphrin IX and negligible levels of protoporphyrin IX and deuteroporphyrin IX. By contrast, in the harderian gland, protoporphyrin IX is the principal porphyrin. In the kidney, uroporphyrin III and coproporphyrin I are most

Table 2. [^{3}H]PK11195 binding inhibitory activity from rat organs

Perfused organ	Inhibitory activity, units/g
Spleen	37,879
Harderian gland	7,407
Kidney	1,508
Liver	1,310
Adrenal gland	583
Brain	<2

Perfused rat tissues were extracted in acidic methanol and purified on C_{18} Sep-Paks; they were then assayed for inhibition of 1 nM [^{3}H]PK11195 binding to rat kidney mitochondria. Crude acidic methanol extracts yielded similar levels of inhibitory activity per g of original wet weight. The quantity of extract producing 50% inhibition in standard [^{3}H]PK11195 binding assays was designated 1 unit. Data are means of two determinations that varied by <20%.

Table 3. Inhibition of [^{3}H]PK11195 binding to rat kidney mitochondria by porphyrins and related compounds

Compound	K_i, nM
Protoporphyrin IX	14.5 ± 10.7
Mesoporphyrin IX	23.1 ± 8.2
Deuteroporphyrin IX	31.3 ± 2
Hemin	40.6 ± 13.7
Hematoporphyrin IX	500 ± 151
Coproporphyrin III	1511 ± 636
Biliverdin	4111 ± 1343

[^{3}H]PK11195 binding to mitochondria was assayed with varying concentrations of inhibitors. Coproporphyrin I and bilirubin were inactive at 10 μM, whereas conjugated bilirubin, cyanocobalmin, δ-aminolevulinic acid, porphobilinogen, and uroporphyrin I were inactive at 0.35 mM. All compounds listed in the table were inactive (K_i > 10,000 nM) in assays of central-type benzodiazepine receptors assayed with [^{3}H]Ro 15-1788. Data are means ± SEM of three to nine determinations.

abundant with somewhat lower concentrations of deuteroporphyrin IX. The only abundant porphyrin in the adrenal gland is deuteroporphyrin IX.

Based on the known potencies of the various porphyrins in competing for mitochondrial benzodiazepine binding and the tissue content of these substances, we calculate that essentially all of the activity of the six organ extracts can be accounted for by their porphyrin content.

DISCUSSION

The major finding of this study is that porphyrins are endogenous ligands for the mitochondrial benzodiazepine receptor. Purification to homogeneity of the inhibitory activity from blood yields spectrophotometrically pure hemin. The inhibitory activity of several tissue extracts also can be accounted for fully by their porphyrin content of protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX, and hemin. The isolated porphyrins display inhibition constants in the nanomolar range. This high affinity is selective for the mitochondrial receptor with less than 1/1000 times as much affinity at central benzodiazepine receptors. The structure activity profile of porphyrins and metalloporphyrins in inhibiting the mitochondrial receptor indicates that the number of carboxylic acids is critical for affinity. Dicarboxylic acid porphyrins display nanomolar potencies, whereas tetracarboxylic acids are very weak and octacarboxylic acids are inactive. Precursors of porphyrin biosynthesis and degradation products are weak or inactive.

Other attempts to purify small molecular weight inhibitors of mitochondrial benzodiazepine receptor have not described purification to homogeneity so it is difficult to assess whether any of the partially purified extracts in the literature contain porphyrins (16–20). However, some of the reports described active fractions with molecular weights similar to porphyrins (16, 20), stability characteristics similar to porphyrins (19, 20), and similar chromatographic properties (16, 19). A partially purified material that inhibits monoamine oxidase as well as mitochondrial benzodiazepine receptors, designated tribulin, seems to have molecular weight, extraction, and chromatographic properties similar to those of porphyrins (20).

Our extraction procedure precipitates proteins so that we would not be able to detect protein ligands for benzodiazepine receptors. A 10,000- to 15,000-dalton protein isolated from various tissues, designated diazepam binding inhibitor or endozepine, inhibits benzodiazepine receptor binding (27, 28). This protein displays micromolar affinity for mitochondrial benzodiazepine receptors, or less potency than some porphyrins by a factor of 100–500. Moreover, the potency of the protein at central-type receptors is similar to its effects on the mitochondrial receptor (29).

What might be the physiological role of porphyrins in regulating mitochondrial benzodiazepine receptors? First, one must consider the potential biological role of mitochondrial benzodiazepine receptors. These sites are localized to the outer membrane of mitochondria (14, 15). Recently, we photoaffinity labeled these receptors with flunitrazepam and identified two discretely labeled NaDodSO$_4$/PAGE bands of 30,000 and 36,000 daltons (30). The 36,000-dalton band corresponds to the voltage-dependent anion channel (VDAC), also designated porin. Porin is a relatively nonselective VDAC that admits molecules with molecular masses of <1000 daltons (31).

How might porphyrins function within mitochondria in association with the benzodiazepine receptor on the outer membrane? The initial and final steps in porphyrin biosynthesis take place within the mitochondria so that the porphyrin precursors of protoporphyrin IX and heme must traverse the outer mitochondrial membrane, conceivably by way of the anion channel of porin. Cytochromes all contain porphyrins and are localized in the outer and inner mitochondrial membranes. Cytochromes selectively associated with the outer mitochondrial membrane include enzymes involved in steroidogenesis. In this connection, it is striking that steroid-forming tissues such as the adrenal gland and testes possess some of the highest levels of mitochondrial benzodiazepine receptors selectively localized to the steroid-forming zona glomerulosa and Leydig cells of the adrenal gland and testes, respectively (1).

Several cytosolic proteins possess porphyrins as prosthetic groups. Examples include hemoglobin, myoglobin, catalase, tryptophan pyrrolase, and several peroxidases. Their biosynthesis requires the transport of porphyrins out of the mitochondria to link with the protein being formed in the extramitochondrial compartments.

Table 4. Porphyrin content of perfused rat organs

Organ	pmol/g of wet weight						
	Proto-porphyrin IX	Meso-porphyrin IX	Deutero-porphyrin IX	Hemin	Hemato-porphyrin IX	Copro-porphyrin I	Uro-porphyrin III
Spleen	<1	353	<1	110,000	<1	<1	<1
Harderian gland	30,650	<1	<1	<300	<1	35	<1
Kidney	<1	<1	67	2,050	<1	211	185
Liver	134	<1	50	<100	<1	<1	<1
Adrenal gland	<1	<1	414	<600	<1	<1	<1
Brain	50	<1	<1	<100	665	<1	89

Organs from perfused rats were dissected, extracted, and loaded on C_{18} Sep-Paks. The eluates were analyzed by HPLC for their porphyrin content (24) using purified porphyrins as standards. Hemin was determined using absorbance at 400 nm and all others were measured by fluorescence. Data are means of two determinations that varied by <15%. In HPLC eluates of the harderian gland, a peak apparently corresponding to harderoporphyrin was observed but not quantitated due to a lack of an appropriate pure standard.

We thank Richard Trifiletti, Richard Nakashima, and Peter Pedersen for helpful discussions and Nancy Bruce for manuscript preparation. This research was supported by Public Health Service Grants DA-00266 and NS-16375, Research Service Award DA-00074 (to S.H.S.), and Training Grant GM-07309 (to J.S.N.). A.V. is supported by the American Heart Association.

1. Anholt, R. R. H. (1986) *Trends Pharmacol. Sci.* **7,** 506–511.
2. Clarke, G. D. & Ryan, P. J. (1980) *Nature (London)* **287,** 160–161.
3. Wang, J. K. T., Morgan, J. I. & Spector, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 753–756.
4. Wang, J. K. T., Morgan, J. I. & Spector, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3770–3772.
5. Zavala, F., Haumont, J. & Lenfant, M. (1985) *Eur. J. Pharmacol.* **103,** 561–566.
6. Ruff, M. R., Pert, C. B., Weber, R. J., Wahl, L. M., Wahl, S. M. & Paul, S. M. (1985) *Science* **229,** 1281–1283.
7. Mestre, M., Bouetard, G., Uzan, A., Gueremy, C., Renault, C., Dubroeucq, M. C. & Le Fur, G. (1985) *Eur. J. Pharmacol.* **112,** 257–260.
8. Mestre, M., Carriot, T., Berlin, C., Uzan, A., Renault, C., Dubroeucq, M. C., Gueremy, C., Doble, A. & Le Fur, G. (1985) *Life Sci.* **36,** 391–400.
9. Mestre, M., Carriot, T., Neliat, G., Uzan, A., Renault, C., Dubroeucq, M. C., Gueremy, C., Dobel, A. & Le Fur, G. (1986) *Life Sci.* **39,** 329–339.
10. Weissman, B. A., Cott, J., Jackson, J. A., Bolger, G. T., Weber, K. H., Horst, W. D., Paul, S. M. & Skolnick, P. (1985) *J. Neurochem.* **44,** 1494–1499.
11. Benavides, J., Guilloux, F., Allam, D. E., Uzan, A., Mizoule, J., Renault, C., Dubroeucq, M. C., Gueremy, C. & Le Fur, G. (1984) *Life Sci.* **34,** 2613–2620.
12. Weiss, S. R. B., Post, R. M., Patel, J. & Marangos, P. J. (1985) *Life Sci.* **36,** 2413–2419.
13. Curran, T. & Morgan, J. I. (1985) *Science* **229,** 1265–1268.
14. Anholt, R. R. H., Pedersen, P. L., De Souza, E. B. & Snyder, S. H. (1986) *J. Biol. Chem.* **261,** 576–583.
15. Anholt, R. R. H., Aebi, U., Pedersen, P. L. & Snyder, S. H. (1986) *Biochemistry* **25,** 2120–2125.
16. Mantione, C. R., Weissman, B. A., Goldman, M. E., Paul, S. M. & Skolnick, P. (1984) *FEBS Lett.* **176,** 69–74.
17. Beaumont, K., Cheung, A. K., Geller, M. L. & Fanestil, D. D. (1983) *Life Sci.* **33,** 1375–1384.
18. Clow, A., Glouer, V., Armando, I. & Sandler, M. (1983) *Life Sci.* **33,** 735–741.
19. Wildmann, J., Niemann, J. & Matthali, H. (1986) *J. Neural Transm.* **66,** 151–160.
20. Elsworth, J. D., Dewar, D., Glover, V., Goodwin, B. L., Clow, A. & Sandler, M. (1986) *J. Neural Transm.* **67,** 45–56.
21. Perry, D. M. & Pedersen, P. L. (1984) *J. Biol. Chem.* **259,** 8917–8923.
22. Hunkeler, W., Mohler, H., Pieri, L., Polc, P., Bonetti, E. P., Cumin, R., Schaffner, R. & Haefely, W. (1981) *Nature (London)* **290,** 514–516.
23. Jensen, J. (1963) *J. Chromatogr.* **10,** 236.
24. Kennedy, S. W., Wigfield, D. C. & Fox, G. A. (1986) *Anal. Biochem.* **157,** 1–7.
25. Schoemaker, H., Bolger, R. G., Horst, D. & Yamamura, H. I. (1983) *J. Pharmacol. Exp. Ther.* **225,** 61–69.
26. Weissman, B. A., Bolger, G. T., Isaac, L., Paul, S. M. & Skolnick, P. (1984) *J. Neurochem.* **42,** 969–975.
27. Guidotti, A., Forchetti, C. M., Corda, M. G., Konkel, D., Bennett, C. D. & Costa E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3531–3535.
28. Shoyab, M., Gentry, L. E., Marquadt, H. & Todaro, G. J. (1986) *J. Biol. Chem.* **261,** 11968–11973.
29. Guidotti, A., Santi, M. R., Berkovich, A., Ferrarese, C. & Costa, E. (1986) *Clin. Neuropharmacol.* Suppl. 4, **9,** 217–225.
30. Trifiletti, R. R., Verma, A. & Snyder, S. H. (1986) *Neurosci. Abstr.* **12,** 666.
31. De Pinto, V., Tommasino, M., Benz, R. & Palmieri, F. (1985) *Biochim. Biophys. Acta* **813,** 230–242.