ELSEVIER

European Journal of Pharmacology 294 (1995) 601–607

ejp

Peripheral benzodiazepine receptor ligands in rat liver mitochondria: effect on cholesterol translocation

Virginia Tsankova [b], Armando Magistrelli [a], Lavinia Cantoni [a], Maria Teresa Tacconi [a,*]

[a] *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milano, Italy*
[b] *Higher Medical School, Faculty of Pharmacy, Sofia, Bulgaria*

Received 10 April 1995; revised 20 September 1995; accepted 22 September 1995

Abstract

Peripheral benzodiazepine receptors mediate cholesterol translocation between the outer and inner mitochondrial membranes in steroidogenic tissues. They are found in many other tissues too, including liver. We studied the effect of the peripheral benzodiazepine receptor ligands PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)isoquinoline-3-carboxamide], Ro 5-4864 (4-chlorodiazepam), hemin, protoporphyrin IX and *N*-methyl protoporphyrin IX on cholesterol mitochondrial intermembrane transport of cholesterol in vitro in rat liver. Endogenous cholesterol translocation from outer to inner mitochondrial membranes was significantly increased by PK11195 and *N*-methyl protoporphyrin IX (140% and 150% increase, respectively, at 1 μM, $P < 0.01$). 5 μM protoporphyrin IX, 1 μM Ro 5-4864 and 5 μM hemin was ineffective. When mitochondria were labeled with exogenous [4-^{14}C]cholesterol, PK11195 and *N*-methyl protoporphyrin IX were the most effective in increasing total cholesterol incorporation and cholesterol translocation into inner membranes, and their effect was dose-dependent. These data suggest that in liver the binding to peripheral benzodiazepine receptors is related to cholesterol translocation and the interaction of ligands with these receptors may play a role in the complex mechanism of regulation of cholesterol traffic between liver mitochondrial membranes.

Keywords: Benzodiazepine receptor, peripheral; Mitochondria, liver; Cholesterol; Porphyrin

1. Introduction

The benzodiazepines are a large class of compounds with anxiolytic, anticonvulsant and sedative effects. They interact with specific receptors in the nervous system, located on neuronal plasma membranes in relationship with GABA receptors and chloride channels (Costa et al., 1975; Richards et al., 1986). The fact that these drugs have other binding sites in many peripheral tissues, but also in the brain (Anholt, 1986; Krueger, 1991; Parola et al., 1993) has raised questions about the physiological functions of peripheral benzodiazepine receptors. The key to a better understanding of the role of peripheral benzodiazepine receptors might be their tissue distribution, subcellular localization and ligand identification.

Peripheral benzodiazepine receptors are distributed in several glandular and secretory tissues, such as adrenal glands, salivary glands, epithelium, testes, kidney, lung and liver (Anholt, 1986; Parola et al., 1993). Receptor densities are highest in the mitochondrial outer membranes (Anholt et al., 1986; Hirsch et al., 1988) suggesting they may be involved in mitochondrial functions. The best known role to date is in steroidogenic tissues, where peripheral benzodiazepine receptors mediate intramitochondrial cholesterol transport, the rate-limiting step in steroid biosynthesis (Mukhin et al., 1989; Papadopoulos et al., 1990; Krueger and Papadopoulos, 1990). Effects on electrolyte equilibrium, calcium transport, mitochondrial respiration, the immune system and cell growth have been suggested

* Corresponding author. Tel.: 39. 2. 39014.1; fax 39. 2. 3546277.

SSDI 0014-2999(95)00603-6

(Parola et al., 1993). In liver peripheral benzodiazepine receptors have been found mainly on the mitochondrial outer membrane (Snyder et al., 1987), though other subcellular localizations are possible (O'Beirne et al., 1990).

Some benzodiazepines, Ro 5-4864 [4-chlorodiazepam], diazepam, and structurally related compounds are potent and selective peripheral benzodiazepine receptor ligands (Costa et al., 1975; Parola et al., 1993). The exogenous ligands also comprise 2-phenylquinoline carboxamides (PK11195 series), imidazo[1,2-*a*]pyridine-3-acetamides (Alpidem series) and pyridazine derivatives (Bourguignon, 1993). Some endogenous compounds like dicarboxylic porphyrins (Snyder et al., 1987; Verma and Snyder, 1988) and diazepam binding inhibitor (Hirsch et al., 1988; Costa and Guidotti, 1991) bind to these receptors with nanomolar and micromolar affinity. Protoporphyrin IX (Snyder et al., 1987; Cantoni et al., 1992) and *N*-methyl protoporphyrin IX are potent inhibitors of [^{3}H]PK 11195 binding in rat liver in vitro and in vivo and *N*-methyl protoporphyrin IX has 20 times higher affinity for peripheral benzodiazepine receptors than protoporphyrin IX (Cantoni et al., 1992).

Porphyrins are mainly synthetized in the liver, the initial and final steps of protoporphyrin IX synthesis, in particular, occurring within liver mitochondria (Tait, 1978). In liver mitochondria cholesterol and various hydroxysterols undergo further oxidation to bile acids (Björkhem and Gustafsson, 1973; Ayaki et al., 1989; Okuda, 1994). The enzymes that catalyze oxidation of the sterol side-chain are on the inner mitochondrial membranes and cholesterol needs a transport system to cross these membranes (Liscum and Dahl, 1992). We still do not fully understand the mechanism cells use to transport cholesterol from extramitochondrial stores to mitochondria and between mitochondrial membranes.

In this study we investigated whether peripheral benzodiazepine receptors play a role in intramitochondrial cholesterol transport in the liver and characterized the effect of both endogenous (protoporphyrin IX, hemin and *N*-methyl protoporphyrin IX) and exogenous ligands, PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-isoquinoline-3-carboxamide] and Ro 5-4864, on the incorporation of cholesterol into mitochondria and its translocation between rat liver outer and inner mitochondrial membranes.

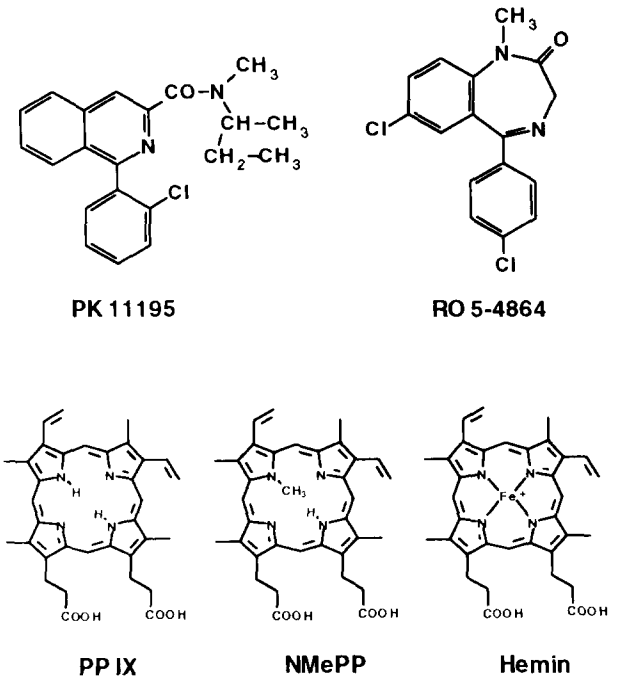

Fig. 1. Chemical structures of peripheral benzodiazepine receptor ligands used in this study. PP = protoporphyrin IX; NMePP = *N*-methyl protoporphyrin IX.

2. Materials and methods

2.1. Materials

[4-^{14}C]Cholesterol (specific activity 52 mCi/mmol) was obtained from The Radiochemical Centre (Amersham, Bucks., UK). Ro 5-4864 was purchased from Fluka (Buchs, Switzerland), PK11195 from Sigma Chemical Co. (St. Louis, MO, USA). Hemin, protoporphyrin IX and *N*-methyl protoporphyrin IX were obtained from Porphyrin Products (Logan, UT, USA). Fig. 1 shows the chemical structures of the peripheral benzodiazepine receptor ligands. All other materials were obtained from standard sources.

A solution of hemin was prepared dissolving 1.3 mg of hemin in 2.5 ml of NaOH 0.1 N containing 12 mg Tris; the volume was brought to 8 ml with distilled water and the pH was adjusted to 7.4 with HCl. Solutions of protoporphyrin IX and *N*-methyl protoporphyrin IX were prepared as follows: 1.5 mg (protoporphyrin IX) or 0.5 mg (*N*-methyl protoporphyrin IX) was dissolved in 100 μl of 1 M KOH; 100 μl of 1 M Tris-chloride buffer (pH 7.8) and 3.7 ml of distilled water were added, and the pH of the solutions was adjusted to 7.4 with HCl. The solutions were filtered through a sterile 0.2 μm filter (Sartorius, SM 17597). Aliquots of filtered solutions were used to calculate the exact concentration (Falk, 1964; De Matteis et al., 1982). Porphyrin solutions were always used within 4 h of preparation.

2.2. Animals

Adult male Sprague-Dawley rats (200–250 g) were obtained from Charles River Italia (Calco, Italy). They were exposed to a 12 h dark/light cycle and were

allowed free access to food and water. Procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with national and international laws and policies (EEC Council directive 86/609, OJ L 358, 1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985.)

2.3. Preparation of mitochondria and subfractionation of mitochondrial membranes

Animals were killed by decapitation and livers were gently homogenized in 0.25 M sucrose, 5 mM Tris-chloride buffer, pH 7.2 (buffer A) (1 g liver in 5 ml buffer) using a Potter-Elvehjem homogenizer. Homogenate was centrifuged at $800 \times g$ for 10 min, and supernatant was recovered and centrifuged again at $9000 \times g$ for 10 min. The resulting pellets were washed, suspended in 0.28 M sucrose, 0.1 mM EDTA, 1.0 mM Tris-chloride buffer, pH 7.2 (buffer B) and spun at $1500 \times g$ for 10 min. The supernatants were carefully decanted and centrifuged at $9000 \times g$ for 10 min, yielding an enriched mitochondrial preparation. All preparations were done on ice and the solutions were kept at 4°C.

Mitochondrial membranes were disrupted, and inner and outer membrane subfractions were prepared by the large amplitude swelling method of Tipton (1967). The mitochondria were suspended slowly in the swelling medium, 20 mM phosphate buffer, pH 7.4 containing 0.02% bovine serum albumin to reduce inner membrane breakage during swelling. Then they were swollen for 20 min and centrifuged by differential centrifugation at $35\,000 \times g$ for 20 min. The mitochondrial membranes were again suspended in the swelling medium. The swollen inner membrane 'ghosts' were removed by spinning at $1900 \times g$ for 15 min and resuspended in buffer A. The supernatant was removed carefully, avoiding suspended material. Outer membranes were sedimented by spinning at $35\,000 \times g$ for 20 min and suspended in buffer A.

2.4. Cholesterol translocation assay

Cholesterol translocation between outer and inner mitochondrial membranes was determined according to Krueger and Papadopoulos (1990). Mitochondria (50 mg protein) were suspended in ice-cold buffer A, pH 7.2, in a final volume of 1 ml. The translocation was started by addition of ligands for peripheral benzodiazepine receptors prepared as described above, followed by incubation at 37°C for 15 min. The concentrations used were 1 μM for PK11195 and Ro 5-4684 and 5 μM for protoporphyrin IX, *N*-methyl protoporphyrin IX and hemin. Reactions were stopped by rapid cooling to 4°C and addition of 8 ml ice-cold buffer. Outer and inner mitochondrial membranes were then prepared. The cholesterol content associated with outer and inner membranes was measured after incubation for 15 min at 37°C (Omodeo Salè et al., 1984) and after lipid extraction (Folch et al., 1956).

In some experiments mitochondria equivalent to 50 mg protein were incubated with 10 μCi [4-^{14}C]cholesterol (0.2 mM) for 15 min at 37°C with or without peripheral benzodiazepine receptor ligands in a final volume of 1 ml. After incubation they were washed with ice-cold buffer A, pH 7.2, to eliminate excess labeled cholesterol and spun down immediately at $9000 \times g$ for 10 min. Inner and outer membranes were then prepared as above. The incorporation of [4-^{14}C]cholesterol into mitochondria and its enrichment into outer and inner membranes was measured by counting the lipid-bound radioactivity, using a Beckman Liquid Scintillation Counter LS 1701.

2.5. Other tests

NADPH cytochrome *c* reductase activity was measured in liver homogenate, microsomes and mitochondria according to Nervi et al. (1980). The activity of cytochrome *c* oxidase, a marker enzyme for the inner mitochondrial membrane, was measured (Smith, 1955). Type B-monoamine oxidase was used as a marker for

Table 1
Distribution and specific activity of marker enzymes in submitochondrial fractions of rat liver

Fractions	Cytochrome *c* oxidase		Monoamine oxidase	
	Specific activity (nmol/min per mg protein)	Recovery (%)	Specific activity (μmol/min per mg protein)	Recovery (%)
Total mitochondria	196.5 ± 9.3		1.21 ± 0.10	
Outer membranes	26.1 ± 3.0	8.42	1.80 ± 0.10	79.60
Inner membranes	270.3 ± 22.1	91.58	0.46 ± 0.08	20.40

Mitochondria were prepared and the outer and inner mitochondrial membranes were separated. The activities of cytochrome *c* oxidase and monoamine oxidase, marker enzymes for inner and outer mitochondrial membranes, were measured. Results are means ± S.E.M. of four separate preparations.

the outer mitochondrial membrane (Schnaitman et al., 1967).

Proteins were measured according to Lowry et al. (1951).

2.6. Statistics

Analysis of variance (ANOVA) was used for statistical analysis; Dunnett's test for multiple comparisons was used when F was significant.

3. Results

3.1. Purity of mitochondrial preparation

To ascertain that the mitochondrial preparation was free of microsomal contamination, the activity of NADPH cytochrome c reductase, a marker enzyme for the endoplasmic reticulum, was measured in whole liver homogenate, microsomes and mitochondria. Specific activity expressed as μmol/min per mg protein was as follows: homogenate 18.2 ± 3.1; mitochondria 1.7 ± 0.5, and microsomes 68.4 ± 14.5. The contamination of mitochondria by microsomes was less than 2% as calculated by comparison of the specific activities.

To study the effect of peripheral benzodiazepine receptor ligands on cholesterol translocation between outer and inner membranes, mitochondrial fractions were separated by large amplitude swelling and subsequent centrifugation. To assess the purity of the preparations, monoamine oxidase and cytochrome c oxidase activities were determined (Table 1). Approximately 92% of the cytochrome c oxidase activity remained in the inner membrane fraction; about 80% of the monoamine oxidase activity was detected in the outer membrane, as expected. When the mitochondria were incubated with peripheral benzodiazepine receptor ligands, no differences in the pattern of enzymatic activities in comparison to controls were observed after the subfractionation.

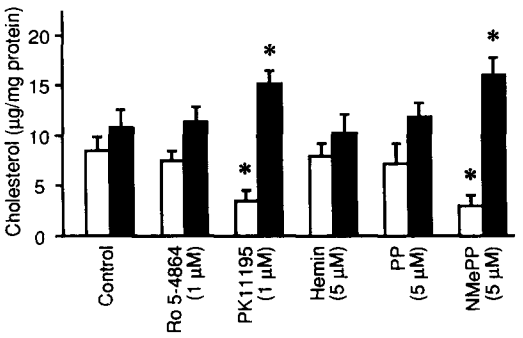

Fig. 2. Effect of peripheral benzodiazepine receptor ligands on cholesterol translocation between outer (open bars) and inner (filled bars) mitochondrial membranes. Mitochondria were incubated for 15 min at 37°C with and without Ro 5-4864, PK11195, hemin, protoporphyrin IX (PP) and N-methyl protoporphyrin IX (NMePP); inner and outer membranes were then prepared, and their cholesterol contents were measured. The results are means ± S.E.M. of four replicates from two representative experiments ($n = 8$). Each experiment was repeated at least four times. $^*P < 0.01$ vs respective controls at 37°C.

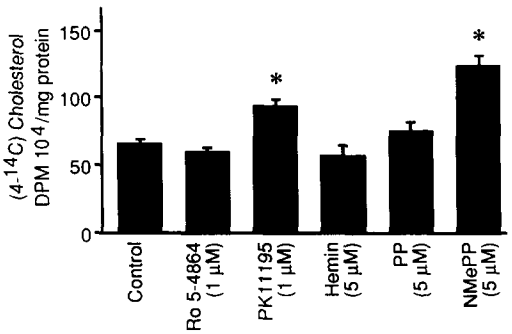

Fig. 3. [4-^{14}C]Cholesterol incorporation in rat liver mitochondria. Mitochondria were incubated for 15 min at 37°C in the presence of [4-^{14}C]cholesterol (10 μCi) with and without peripheral benzodiazepine receptor ligands. Mitochondria were then washed with 0.25 M sucrose, 0.5 mM Tris-chloride buffer, pH 7.2, spun down at $9000 \times g$, and labeled cholesterol incorporated was measured. The results are means ± S.E.M. of duplicates from four experiments ($n = 8$). For abbreviations, see Fig. 1. $^*P < 0.01$ vs. controls.

3.2. Endogenous cholesterol translocation in rat liver mitochondria

Fig. 2 illustrates the effect of peripheral benzodiazepine receptor ligands on cholesterol translocation in rat liver mitochondria with no exogenous cholesterol. After a 15 min incubation at 37°C, the ratio between inner membrane and outer membrane cholesterol content was 1.24 ± 0.15. When the mitochondria were incubated with peripheral benzodiazepine receptor ligands, the following results were obtained: 1 μM PK11195 and 5 μM N-methyl protoporphyrin IX increased cholesterol translocation (inner/outer ratios were 4.6 ± 0.5 and 5 ± 0.3, $P < 0.01$ in comparison to control); 1 μM Ro 5-4864, protoporphyrin IX and hemin (5 μM) did not affect translocation (ratios were no different from control, 1.5 ± 0.08, 2.0 ± 0.13 and 1.28 ± 0.08, respectively). The increase in inner membrane cholesterol content was accompanied by a concominant decrease in outer membrane cholesterol, as expected, since no exogenous cholesterol was added in these experiments.

The degree of possible contamination of inner membranes with outer membranes (approximately 20%) during preparation was considered not to affect the results, since the changes in the ratio of cholesterol content in inner and outer membranes after translocation were of a much greater order of magnitude (approximately 350% and 400% for PK11195 and N-methyl protoporphyrin IX respectively).

3.3. Incorporation and translocation of [4-^{14}C]cholesterol into mitochondria

To clarify whether peripheral benzodiazepine receptor ligands affected the incorporation of exogenous cholesterol into mitochondria, the following experiment was done. Mitochondria were incubated in the presence of [4-^{14}C]cholesterol with and without peripheral benzodiazepine receptor ligands. After washing to eliminate the excess of cholesterol, samples were used to measure cholesterol incorporation into total mitochondria (Fig. 3). After 15 min incubation at 37°C, the levels of cholesterol incorporated into mitochondria in the presence of PK11195 (1 μM) and *N*-methyl protoporphyrin IX (5 μM) were increased to 142% and 195%, respectively in comparison to control; protoporphyrin IX (5 μM), hemin (5 μM) and Ro 5-4864 (1 μM) had no effect.

Subsequently, to determine the effect of peripheral benzodiazepine receptor ligands on the distribution of exogenous cholesterol between mitochondrial membranes, cholesterol-enriched mitochondria were subfractionated and labeled cholesterol was measured in inner and outer mitochondrial membranes (Fig. 4). After a 15 min incubation at 37°C the inner membrane/outer membrane ratio of labeled cholesterol was 3.73 ± 0.4 in control mitochondria. This ratio was 5.28 ± 0.75 in the presence of PK11195 (1μM); *N*-methyl protoporphyrin IX raised the ratio at both 1 μM and 5 μM (5.0 ± 0.6 and 5.9 ± 0.4, respectively). Ro 5-4864 (1 μM) and protoporphyrin IX (5 μM) were not active. Hemin (5 μM) actually tended to cause a decrease (ratio 2.5 ± 0.2).

The effect on cholesterol translocation of PK11195 and *N*-methyl protoporphyrin IX was dose-dependent

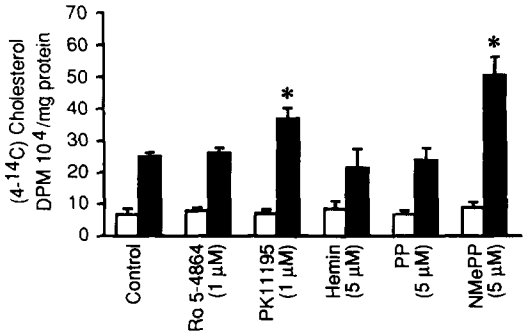

Fig. 4. Effect of peripheral benzodiazepine receptor ligands on [4-^{14}C]cholesterol distribution between outer (open bars) and inner (filled bars) mitochondrial membranes. Outer and inner membranes from [4-^{14}C]cholesterol labeled mitochondria, incubated with and without peripheral benzodiazepine receptor ligands, were prepared as described and labeled cholesterol was measured in each subfraction. The results are means ± S.E.M. of duplicates from five experiments (n = 10). For abbreviations, see Fig. 1. $^*P < 0.01$ vs. respective controls.

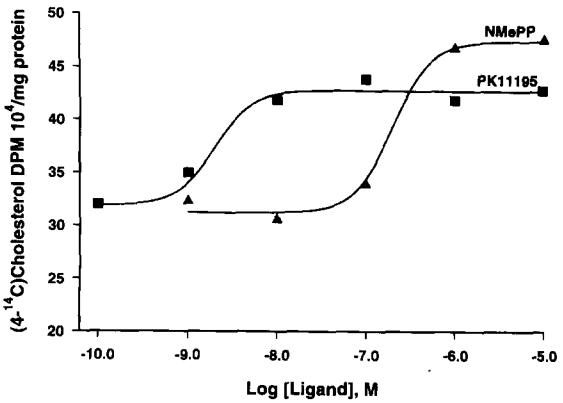

Fig. 5. Effect of PK11195 and *N*-methyl protoporphyrin IX (NMePP) on inner membrane [4-^{14}C]cholesterol content: concentration-response curve. Mitochondria were incubated for 15 min at 37°C in the presence of [4-^{14}C]cholesterol (10 μCi) with PK11195 and *N*-methyl protoporphyrin IX used in the concentration range from 10^{-10} to 10^{-5} M. Outer and inner membranes were separated and the [4-^{14}C]cholesterol content associated with the membrane fractions was measured. Values are means of duplicates from two experiments (n = 4). Curves were generated by computer-assisted non-linear regression analysis, according to a least-squares curve-fitting program.

(Fig. 5). The compounds were tested in the concentration range from 10^{-10} to 10^{-5} M: the half-maximal effect of PK11195 was in the nanomolar range, but it was in the micromolar range for *N*-methyl protoporphyrin IX. Under conditions of receptor saturation, *N*-methyl protoporphyrin IX was more effective than PK11195 on cholesterol translocation.

4. Discussion

Cholesterol is a nonpolar compound and needs a transport system to cross mitochondrial membranes (Liscum and Dahl, 1992; Trotter and Voelker, 1994). At present the mechanism used by cells to transport cholesterol from extramitochondrial stores to mitochondria and inside is still not known. Besides the suggestion of vesicular transport between membranes and transport by protein carriers like sterol carrier protein$_2$ (Reinhart, 1990), a role for peripheral benzodiazepine receptors has been proposed in steroidogenic tissues (Mukhin et al., 1989; Papadopoulos et al., 1990).

We found that in rat liver ligands for peripheral benzodiazepine receptors facilitated cholesterol incorporation and its translocation from outer to inner mitochondrial membranes. Our results suggest that, as in the adrenals, in the liver too binding to peripheral benzodiazepine receptors might affect cholesterol translocation. The rank order of potency of the effects of the ligands was *N*-methyl protoporphyrin IX > PK11195 > protoporphyrin IX > Ro 5-4864 ≫ hemin.

In accordance with the binding affinities, the ED_{50} for the most potent compounds was in the nanomolar range (PK11195) and in the micromolar range (*N*-methyl protoporphyrin IX), suggesting that the effect on cholesterol translocation is connected with an interaction with the receptor.

The effect of exogenous ligands on cholesterol translocation was closely related to their binding affinities with the receptors in the liver. PK11195, which binds the receptor with higher affinity ($IC_{50} < 2.5$ nM) (O'Beirne et al., 1990), was more potent than Ro 5-4864 ($IC_{50} > 2.5$ nM) in stimulating the translocation of both endogenous and added cholesterol when both ligands were tested at the same concentration.

Data based on thermodynamic analysis suggest that PK11195 is an antagonist, whereas Ro 5-4864 is an agonist of these receptors (Mizoule et al., 1985). PK11195 antagonizes the action of Ro 5-4864 both in vitro in chemotaxis of human monocytes (Ruff et al., 1985) and in vivo in shock-induced suppression of drinking in rats (Slobodyansky et al., 1989). The potency of Ro 5-4864 as a peripheral benzodiazepine receptor ligand varies markedly in different organs and species (Awad and Gavish, 1987), whereas the effect of PK11195 is relatively constant.

The structure of porphyrins influences their affinity for peripheral benzodiazepine receptors. In kidney mitochondria Snyder et al. (1987) and Verma and Snyder (1988) observed substantial differences in binding potency depending on the type of substituents in the tetrapyrrole ring. Protoporphyrin IX, *N*-methyl protoporphyrin IX and also hemin, the protoporphyrin IX complex with iron, were the most potent among heme pathway intermediates in binding to peripheral benzodiazepine receptors (with protoporphyrin IX being slightly more potent than the others) (Snyder et al., 1987; Verma and Snyder, 1988).

However, the binding affinities of these compounds do not always correlate with their biological activities. In fact, coproporphyrinogen, which has low affinity for peripheral benzodiazepine receptors, was active in inducing peripheral benzodiazepine receptor-dependent differentiation of mouse erythroleukemia cells (Taketani et al., 1994).

An alternative explanation of the different potencies of the effective ligands can be obtained by looking at their chemical structures. The less polar ligands are the most potent in cholesterol translocation. Both Ro 5-4864 and PK11195 have an imine nitrogen, but in Ro 5-4864 it is protonated at pH 7.2 (as used in our experiments), while in PK11195 it is conjugated with an electron withdrawing carbonyl group that reduces the electron density on the N atom and makes it a weak base; moreover the steric hindrance from the alkyl chain reduces the molecule's polarity. *N*-Methyl protoporphyrin IX is less polar than protoporphyrin IX. Hemin is polar because of the presence of an iron(III) cation. These differences in polarity might affect the receptor structure due to hydrophobic interactions and influence cholesterol transport.

In mitochondria from adrenals and other steroidogenic tissues, cholesterol is translocated before it is metabolized to pregnenolone by cytochrome $P\text{-}450_{scc}$, localized on inner membrane (Krueger and Papadopoulos, 1990). In other tissues, including liver, cholesterol translocation is important for the activity of sterol 27-hydroxylase, which is located in the mitochondrial inner membrane and which has been suggested to have a role in cholesterol detoxification from the periphery (Björkhem et al., 1994).

The effect of porphyrins on cholesterol translocation in liver, described here, might be important to explain the alterations in cholesterol and biliary acid metabolism observed in experimental and human porphyrias (Cantoni et al., 1983; Taddeini et al., 1964). Hypercholesterolemia has been reported in experimental models in which there is massive accumulation of protoporphyrin IX and N-methyl protoporphyrin IX (De Matteis, 1966). It is not known whether porphyrins influence cholesterol translocation under basal conditions, when their levels are kept low by homeostatic mechanisms, nor when the rate of synthesis of heme is boosted by cytochrome P-450 inducers like barbiturates, which cause a small increase in biliary and urinary excretion of porphyrins (Smith and De Matteis, 1980).

It has been suggested that peripheral benzodiazepine receptors may be involved in porphyrin transport inside the mitochondria (Taketani et al., 1995). Interestingly, *N*-methyl protoporphyrin IX, which is more active than protoporphyrin IX on cholesterol translocation, has to migrate inside the mitochondrion to inhibit ferrochelatase (EC. 4.99.1.1), leading to hepatic protoporphyria (De Matteis et al., 1987).

Acknowledgements

The authors thank Dr. Mario Salmona for the critical review of the manuscript and Dr. Enrico Monzani from the Chemistry Department of the University of Pavia for his useful advice on the chemistry properties of peripheral benzodiazepine receptor ligands.

References

Anholt, R.R.H., 1986, Mitochondrial benzodiazepine receptors as potential modulators of intermediary metabolism, Trends Pharmacol. Sci. 7, 506.

Anholt, R.R.H., P.L. Pedersen, E.B. De Souza and S.H. Snyder, 1986, The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane, J. Biol. Chem. 261, 576.

Awad, M. and M. Gavish, 1987, Binding of [^{3}H]Ro 5-4864 and [^{3}H]PK11195 to cerebral cortex and peripheral tissues of various species: species differences and heterogeneity in peripheral benzodiazepine binding sites, J. Neurochem. 49, 1407.

Ayaki, Y., E. Kok and N.B. Javitt, 1989, Cholic acid synthesis from 26-hydroxycholesterol and 3-hydroxy-5-cholestenoic acid in the rabbit, J. Biol. Chem. 264, 3818.

Björkhem, I. and J. Gustafsson, 1973, ω-Hydroxylation of steroid side-chain in biosynthesis of bile acids, Eur. J. Biochem. 36, 201.

Björkhem, I., O. Andersson, U. Diczfalusy, B. Sevastik, R. Xiu, C. Duan and E. Lund, 1994, Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages, Proc. Natl. Acad. Sci. USA 91, 8592.

Bourguignon, J., 1993, Endogenous and synthetic ligands of mitochondrial benzodiazepine receptors: structure-affinity relationships, in: Peripheral Benzodiazepine Receptors, ed. E. Giesen-Crouse (Academic Press, London) p. 59.

Cantoni, L., C. Di Padova, P. Rovagnati, R. Ruggieri, D. Dal Fiume and R. Tritapepe, 1983, Bile secretion and liver microsomal mixed function oxidase system in mice with griseofulvin-induced hepatic protoporphyria, Toxicology 27, 27.

Cantoni, L., M. Rizzardini, M. Skorupska, A. Cagnotto, A. Codegoni, N. Pecora, L. Frigo, C. Ferrarese and T. Mennini, 1992, Hepatic protoporphyria is associated with a decrease in ligand binding for the mitochondrial benzodiazepine receptors in the liver, Biochem. Pharmacol. 44, 1159.

Costa, E. and A. Guidotti, 1991, Diazepam binding inhibitor (DBI): a peptide with multiple biological actions, Life Sci. 49, 325.

Costa, E., A. Guidotti, C.C. Mao and A. Suria, 1975, New concepts on the mechanism of action of benzodiazepines, Life Sci. 17, 167.

De Matteis, F., 1966, Hypercholesterolaemia and liver enlargement in experimental hepatic porphyria, Biochem. J. 98, 23.

De Matteis, F., A.H. Jackson, A.H. Gibbs, K.R.N. Rao, J. Atton, S. Weerasinghe and C. Hollands, 1982, Structural isomerism and chirality of *N*-monosubstituted protoporphyrins, FEBS Lett. 142, 44.

De Matteis, F., A.H. Gibbs and A.E. Holley, 1987, Occurrence and biological properties of N-methyl protoporphyrin, Ann. NY Acad. Sci. 514, 30.

Falk, J.E., 1964, in: Porphyrins and Metalloporphyrins (Elsevier, New York), p. 236.

Folch, J., M. Lees and G.H. Sloane Stanley, 1956, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226, 497.

Hirsch, J.D., C.F. Beyer, L. Malkowitz, C.C. Loullis and A.J. Blume, 1988, Characterization of ligand binding to mitochondrial benzodiazepine receptors, Mol. Pharmacol. 34, 164.

Krueger, K.E., 1991, Peripheral-type benzodiazepine receptors: a second site of action for benzodiazepines, Neuropsychopharmacology 4, 237.

Krueger, K.E. and V. Papadopoulos, 1990, Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells, J. Biol. Chem. 265, 15015.

Liscum, L. and N.K. Dahl, 1992, Intracellular cholesterol transport, J. Lipid Res. 33, 1239.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Mizoule, J., A. Gauthier, A. Uzan, C. Renault, M.C. Dubroeucq, C. Guèrèmy and G. Le Fur, 1985, Opposite effects of two ligands for peripheral type benzodiazepine binding sites, PK11195 and Ro 5-4864, in a conflict situation in the rat, Life Sci. 36, 1059.

Mukhin, A.G., V. Papadopoulos, E. Costa and K.E. Krueger, 1989, Mitochondrial benzodiazepine receptors regulate steroid biosynthesis, Proc. Natl. Acad. Sci. USA 86, 9813.

Nervi, A.M., R.O. Peluffo, R.R. Brenner and A.I. Leikin, 1980, Effect of ethanol administration on fatty acid desaturation, Lipids 15, 263.

O'Beirne, G.B., M.J. Woods and D.C. Williams, 1990, Two subcellular locations for peripheral-type benzodiazepine acceptors in rat liver, Eur. J. Biochem. 188, 131.

Okuda, K., 1994, Liver mitochondrial P450 involved in cholesterol catabolism and vitamin D activation, J. Lipid Res. 35, 361.

Omodeo Salè, F., S. Marchesini, P.H. Fishman and B. Berra, 1984, A sensitive enzymatic assay for determination of cholesterol in lipid extracts, Anal. Biochem. 142, 347.

Papadopoulos, V., A.G. Mukhin, E. Costa and K.E. Krueger, 1990, The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis, J. Biol. Chem. 265, 3772.

Parola A.L., H.I. Yamamura and H.E. Laird II, 1993, Peripheral-type benzodiazepine receptors, Life Sci. 52, 1329.

Reinhart, M.P., 1990, Intracellular sterol trafficking, Experientia 46, 599.

Richards, J.G., P. Schoch, H. Möhler and W. Haefely, 1986, Benzodiazepine receptors resolved, Experientia 42, 121.

Ruff, M.R., C.B. Pert, R.J. Weber, L.M. Wahl, S.M. Wahl and S.M. Paul, 1985, Benzodiazepine receptor-mediated chemotaxis of human monocytes, Science 229, 1281.

Schnaitman, C., V.G. Erwin and J.W. Greenawalt, 1967, The submitochondrial localization of monoamine oxidase, J. Cell. Biol. 32, 719.

Slobodyansky, E., A. Guidotti, C. Wambebe, A. Berkovich and E. Costa, 1989, Isolation and characterization of a rat brain triakontatetraneuropeptide, a posttranslational product of diazepam binding inhibitor: specific action at the Ro 5-4864 recognition site, J. Neurochem. 53, 1276.

Smith, A.G. and F. De Matteis, 1980, Drugs and the hepatic porphyrias, Clin. Haematol. 9, 399.

Smith, L., 1955, Spectrophotometric assay of cytochrome c oxidase, in: Methods of Biochemical Analysis, ed. D. Glick (Interscience, New York) p. 427.

Snyder, S.H., A. Verma and R.R. Trifiletti, 1987, The peripheral-type benzodiazepine receptor: a protein of mitochondrial diethoxycarbonyl-1,4-dihydrocollidine. Role of a porphyrin-like inhibitor of protohaem ferrolyase, Biochem. J. 180, 241.

Taddeini, L., K.L. Nordstrom and J. Watson, 1964, Hypercholesterolemia in experimental and human hepatic porphyria, Metabolism 13, 691.

Tait, G.H., 1978, The biosynthesis and degradation of heme, in: Handbook of Experimental Pharmacology, ed. F. De Matteis and W.N. Aldridge (Springer-Verlag, Berlin, Heidelberg), p. 1.

Taketani, S., H. Kohno, M. Okuda, T. Furukawa and R. Tokunaga, 1994, Induction of peripheral-type benzodiazepine receptors during differentiation of mouse erythroleukemia cells, J. Biol. Chem. 269, 7527.

Taketani, S., H. Kohno, T. Furukawa and R. Tokunaga, 1995, Involvement of peripheral type benzodiazepine receptors in the intracellular transport of heme and porphyrins, J. Biochem. 117, 875.

Tipton, K.F., 1967, The sub-mitochondrial localization of monoamine oxidase in rat liver and brain, Biochim. Biophys. Acta 135, 910.

Trotter, P.J. and D.R. Voelker, 1994, Lipid transport processes in eukaryotic cells, Biochim. Biophys. Acta 1213, 241.

Verma A. and S.H. Snyder, 1988, Characterization of porphyrin interactions with peripheral type benzodiazepine receptors, Mol. Pharmacol. 34, 800.