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Suppression of T_3- and fatty acid-induced membrane permeability transition by L-carnitine

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Abstract

Cytochrome *c* (Cyt. *c*) is known to be released from the mitochondria into the cytosol by means of the membrane permeability transition (MPT) mechanism, thereby activating caspase cascade activity, and inducing cell apoptosis. Recently we reported that L-carnitine suppressed palmitoyl-CoA-induced MPT as well as apoptosis in some cell types (Biochem. Pharmacol, in press). In the present study T_3 was found to induce MPT and Cyt. *c* release, while cyclosporin A (CsA), bovine serum albumin (BSA) and L-carnitine were found to inhibit this action in a concentration-dependent manner. Similarly, long chain fatty acid (LCFA) also induced MPT and Cyt. *c* release, which was then inhibited by CsA, BSA and L-carnitine. From these results the authors postulate that T_3-induced MPT is in part regulated by fatty acid metabolism through a dynamic balance between LCFAs and L-carnitine.

Keywords: Apoptosis mechanism; Cytochrome *c*; L-Carnitine; Long chain fatty acid; Membrane permeability transition; Mitochondria; Triiodothyronine

1. Introduction

Apoptosis, also known as programmed cell death (PCD), is a genetically controlled response for cells to neatly commit suicide, and plays an important role during development as well as in homeostasis (Mignotte and Vayssiere, 1998). Too much apoptosis may result in a variety of diseases, such as neurodegenerative disorder, acquired immunodeficiency syndrome (AIDS) or osteoporosis, while too little apoptosis may result in cancer, autoimmune disease or viral infection (Thompson, 1995). Apoptosis hallmarks include chromatin condensation, protease and endonuclease activation, internucleosomal DNA fragmentation, membrane blebbing and cell shrinkage (Thornberry and Lazebnik, 1998).

In apoptosis, a family of cysteine proteases called caspases participates in a proteolytic cascade, which leads to cell disassembly (effectors),

Abbreviations: ALC, acetyl-L-carnitine; CPTI, carnitine palmitoyltransferase I; CsA, cyclosporin A; diS-C3-(5), 3,3′-dipropylthiodicarbocyanine iodide; MPT, membrane permeability transition; OCTN2, organic cation transporter 2; carnitine, 3-hydroxy-4-*N*-trimethyl-aminobutyric acid; PLA_2, phospholipase A_2; Pi, inorganic phosphate; T_3, triiodothyronine.

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and also functions in upstream regulatory events (initiators) (Alnemri et al., 1996; Thornberry and Lazebnik, 1998).

Mitochondria are thought to be an important executioner of apoptosis. The membrane permeability transition (MPT) is induced by mitochondrial dysfunction, including a decrease in inner membrane potential, respiratory control and cardiolipin levels in the membranes (Zoratti and Szabó, 1995). MPT pore results in a release of the apoptogenic factor cytochrome *c* (Cyt. *c*) from the mitochondria into the cytosol. Once in the cytosol Cyt. *c*, forms a complex of apoptotic protease activating factor (Apafs), composed of Cyt. *c* (Apaf-2), Apaf-1 and procaspase-9 (Apaf-3), leading to the initiation of a caspase cascade, and eventually apoptosis (Li et al., 1997; Green and Reed, 1998; Raff, 1998). MPT pore opening is affected by many chemical compounds, including thyroid hormones (L-thyroxine, T_4, and the more biologically active triiodo-L-thyronine, T_3) (Kalderon et al., 1995; Malkevitch et al., 1997; Castilho et al., 1998; Hermesh et al., 2000). The mechanism involved in pore opening by T_4 and T_3 is not clear, however.

Tail atrophy during anuran metamorphosis is the result of apoptosis, or PCD (Kerr et al., 1974), and is triggered and controlled by thyroid hormones (Tata, 1993; Shi et al., 1996; Shi, 2000). Thus, tadpole tail provides excellent material for the study of the mechanism underlying thyroid hormone-induced pore opening. T_3 induces the proapoptotic gene *Bax* expression and apoptosis in the tail muscle of *Xenopus laevis* tadpoles (Sachs et al., 1997). T_3 treatment also causes *Bax* expression and apoptosis in the optic lobe of the chick embryo (Ghorbel et al., 1997). Bax is found to induce Cyt. *c* release from mitochondria (Reed, 1997). Cyclosporin A (CsA), an MPT inhibitor, prevents T_3-induced regression of bullfrog tadpole tail (Little and Flores, 1992). Moreover, according to Su et al. (1997a,b), T_3 treatment of *X. laevis* tadpole intestinal epithelium cells cultured in vitro resulted in apoptosis, which is inhibitable by CsA (Su et al., 1997b). These findings suggest that thyroid hormone is involved in the induction of MPT in mitochondria. In this context, some investigators reported a CsA-sensitive MPT (Kalderon et al., 1995; Su et al., 1997a; Sultan and Sokolove, 2001), but others reported a CsA-insensitive MPT induced by fatty acids and T_3 (Hermesh et al., 2000; Sultan and Sokolove, 2001). Sensitivity of MPT to CsA is dependent on the concentration of Ca^{2+} (Sultan and Sokolove, 2001). Under concentrations of Ca^{2+} lower than 75 nmol/mg protein free fatty acids induced an MPT that is sensitive to CsA (classic CsA-sensitive MPT, MPT). However, higher concentrations of Ca^{2+} can open an MPT that is insensitive to CsA (non-classic MPT, NCPT). Thus the intracellular concentration of calcium ion also has an important role in T_3-induced MPT. Various investigators have described remarkable differences in the concentration of T_3 required to induce MPT in isolated rat liver mitochondria (Kalderon et al., 1995; Malkevitch et al., 1997; Castilho et al., 1998; Hermesh et al., 2000). These results make it difficult to interpret the effect of thyroid hormone on the mechanism of MPT induction.

Long chain fatty acids (LCFAs) have been shown to promote MPT pore opening, and this action is inhibited in the presence of CsA (Broekemeier and Pfeiffer, 1995; Wieckowski et al., 2000; Arita et al., 2001). A non-metabolizable long chain fatty acyl analogue has a direct effect on mitochondria, which is similar to that exerted by thyroid hormone treatment (Hermesh et al., 2000). Woitczak and Lehninger (1961) reported that free fatty acids are generated during the swelling of mitochondria by treatment with T_3. MPT induced by fatty acids is sensitive not only to CsA but also L-carnitine, a substance important in the translocating of free fatty acids into the mitochondria matrix for β-oxidation (Kanno et al., 2000; Furuno et al., 2001).

The purpose of the present study is to investigate the possible involvement of fatty acid metabolism in T_3-induced MPT, as well as L-carnitine inhibitory action.

2. Materials and methods

2.1. Chemicals

Arachidonic acid, Cyt. *c*, ribonuclease (RNase) A and proteinase K were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Monoclonal antibody against Cyt. *c* was purchased from PharMingen (San Diego, CA, USA). All other chemicals were of analytical grade and were obtained from Nacalai Tesque (Kyoto, Japan).

2.2. Preparation of rat liver mitochondria

Mitochondria were isolated from rat liver essentially by the method of Hogeboom (1955) using sucrose density gradient centrifugation as described previously (Mustafa et al., 1966). Protein concentrations were determined by the method of Bradford (1976), using BSA as a standard.

2.3. Assay for mitochondrial swelling and membrane potential

Mitochondria (0.1 mg protein/ml) were incubated in 10 mM Tris–HCl buffer (pH 7.4) containing 0.15 M KCl (Tris–KCl solution) at 25°C. Large amplitude swelling of the mitochondria, representing MPT (Zoratti and Szabó, 1995), was measured spectrophotometrically at 540 nm at 25°C using a dual beam spectrophotometer (Shimadzu UV-3000) equipped with a thermostatically-controlled cuvette holder and a magnetic stirrer.

For the analysis of membrane potential, mitochondria (0.1 mg protein/ml) were incubated in Tris–KCl solution at 25°C in the presence of 0.1 nM cyanine dye, diS-C3-(5) (Hoffman and Laris, 1974). Then fluorescence intensity was recorded at 672 nm using excitation light at 650 nm in a fluorospectrophotometer (Hitachi 650-10LC) equipped with a thermostatically-controlled cuvette holder (Utsumi et al., 1995).

2.4. Western Blot Analysis

Rat liver mitochondria (0.1 mg protein/ml) were incubated in a 0.15 M KCl medium containing 10 mM Tris–HCl (pH 7.4) at 25°C for 10 min. Supernatant and mitochondrial fractions were obtained by centrifugation at $2000 \times g$ for 10 min. The same incubation medium was then added to the mitochondrial fraction to bring it up to the volume of the supernatant fraction.

Half a milliliter of the supernatant or 0.5 ml of the mitochondrial fraction were added to 0.5 ml volumes of an SDS-sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 10% β-mercaptoethanol, 20% glycerol, and 0.002% bromophenol blue) and then boiled at 100°C for 5 min. Boiled samples were subjected to SDS-polyacrylamide gel electrophoresis. After transfer to Immobilon filters (Millipore Co.), filters were incubated with anti-cytochrome *c* antibody (1:1000 dilution) and then with horseradish peroxidase-linked secondary antibody (1:2000 dilution) and analyzed using an ECLplus kit (Amersham Co.) (Yang and Cortopassi, 1998; Yabuki et al., 2000).

3. Results

3.1. T_3-induced MPT

T_3 induced the opening of MPT pore in the

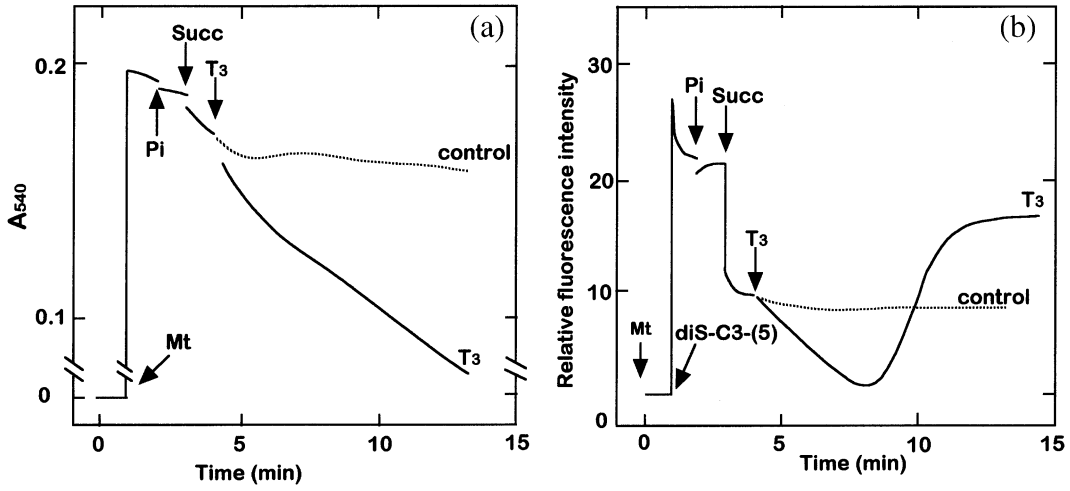

Fig. 1. T_3-induced MPT. Mitochondria (0.1 mg protein/ml) were incubated in Tris–HCl buffer (pH 7.4) containing 0.15 M KCl at 25°C. (A) Mitochondrial swelling was monitored by absorption at 540 nm. (B) Membrane potential was monitored by fluorescence of diS-C3-(5). Reagents used were 5 mM succinate, 2 mM phosphate and 25 μM of T_3 for the measurement of swelling and 0.15 mg/ml diS-C3-(5) for membrane potential. Similar results were obtained in three separate trials.

presence of Ca^{2+} (1 μM) (Castilho et al., 1998). In a medium of 0.15 M KCl-10 mM Tris–HCl buffer (pH 7.4) at 25°C, 25 μM T_3 induced mitochondrial swelling and depolarization of membrane potential in the presence of inorganic phosphate (Pi) and succinate as respiratory substrate (Fig. 1). Mitochondrial swelling was not observed in the presence of ruthenium red, which inhibits mitochondrial Ca^{2+} uptake (data not shown).

3.2. Suppression of T_3-induced MPT by L-carnitine

T_3-induced MPT was found to be concentration-dependent, with maximum swelling of mitochondria seen at a concentration of 50 μM T_3 (Fig. 2a). The minimum concentration of T_3 required to induce mitochondrial swelling was just over 1 μM in the presence of rotenone, Pi and succinate. T_3 concentration required to mitochondrial swelling depends upon the composition of reaction medium containing Ca^{2+} and Pi concentrations as well as mitochondrial concentration, as described in Castilho et al. (1998). These authors incubated rat liver mitochondria (0.3 mg/ml) in reaction medium containing 0.1 mM Pi and 460 μM Ca^{2+} and 500 μM EGTA (free Ca^{2+} concentration, 1 μM), and then 0.5 μM T_3 or 1.0 μM T_3 was added. Both 1 μM T_3 and 20 μM T_3 were required in the present study. T_3-induced MPT was suppressed by L-carnitine in a concentration-dependent manner (Fig. 2b).

3.3. Suppression of T_3-induced MPT by various reagents

In a recent study Waring and Beaver (1996) demonstrated that sensitivity to CsA is the only criterion necessary to distinguish the involvement of MPT in apoptosis. Other investigators, however, have reported T_3-induced mitochondrial swelling that is not only CsA-sensitive (Kalderon et al., 1995; Su et al., 1997a,b; Castilho et al., 1998), but also insensitive (Malkevitch et al., 1997). Thus, we examined the effects of CsA and several other reagents. CsA (2 μM), BSA (2 μM) and chlorpromazine (10 μM) were also found to inhibit T_3-induced MPT in a manner similar to L-carnitine (Fig. 3). However, the inhibitory effects of various reagents, including L-carnitine, were seen to be dependent on the concentration of T_3, and the effects greatly decreased by T_3 concentrations greater than 40 μM. At such high concentrations, thyroxine promotes an increase in CsA-insensitive MPT (Malkevitch et al., 1997).

3.4. T_3-induced Cyt. c release and inhibition by various reagents

Studies have shown that Cyt. *c* is released from the mitochondria during reagent-induced MPT. Fig. 4 shows Cyt. *c* release induced by 25 μM T_3. Cyt. *c* release was suppressed by CsA (2 μM),

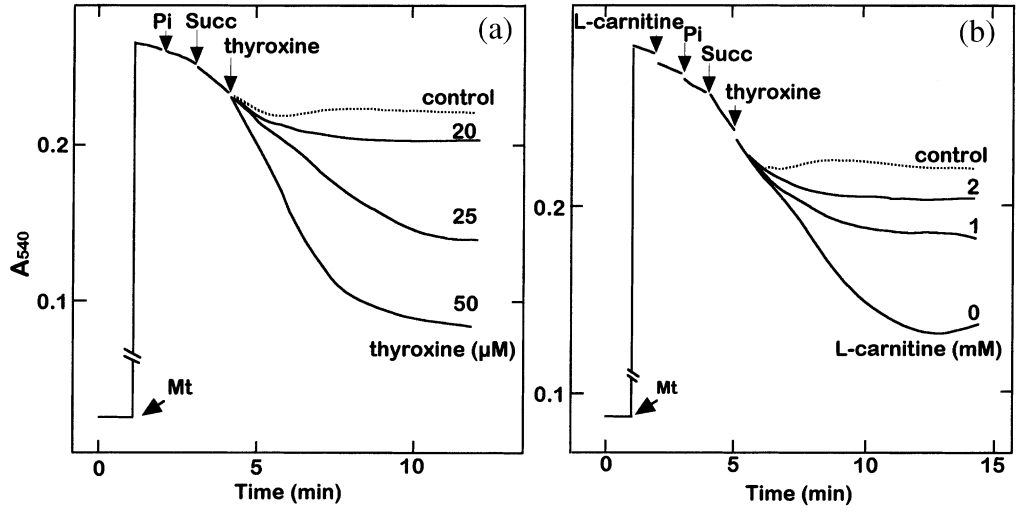

Fig. 2. Suppression of T_3-induced MPT by L-carnitine. Experimental conditions were the same as described for Fig. 1. Various concentrations of L-carnitine were added to the medium before adding mitochondria. (a) shows the dose-dependent swelling of mitochondria at various concentrations of T_3. (b) shows the dose-dependent L-carnitine suppression of mitochondrial swelling induced by 25 μM T_3. Similar results were obtained in three separate trials.

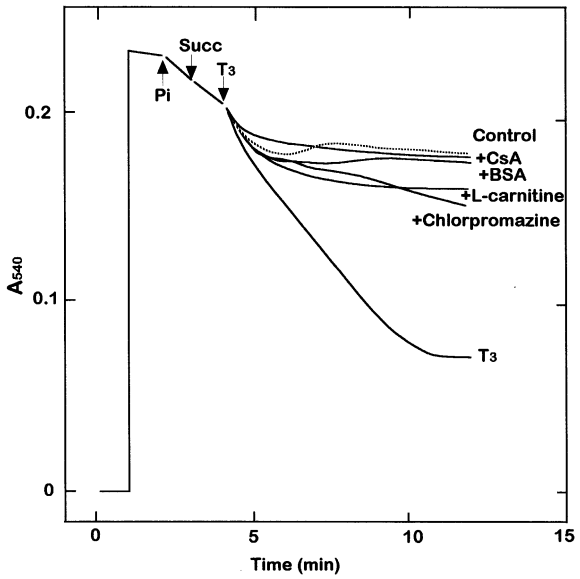

Fig. 3. Suppression of T_3-induced MPT by various reagents. Experimental conditions were as described for Fig. 1. The dotted line (control) shows absorption in the absence of T_3 and the inhibiting reagents. The following concentrations were used: T_3, 25 μM; CsA, 2 μM; BSA, 2 μM; chlorpromazine, 10 μM; L-carnitine, 2 mM.

L-carnitine (1 mM), BSA (0.1 mg/ml) and chlorpromazine (10 μM).

3.5. Arachidonic acid-induced MPT and inhibition by CsA, BSA and L-carnitine

A recent study has shown that the thyromimetic protonophoric activity of non-metabolizable long chain fatty acyl analogue (Medica 16) and the uncoupling activity of T_3 converge onto adenine nucleotide transporter, a component of MPT pore (Hermesh et al., 2000). In this context, we reported in a previous paper that polyunsaturated long chain fatty acids (PUFA) induced MPT in a CsA-sensitive manner (Arita et al., 2001). In the present study we examined the effects of various other reagents on PUFA-induced MPT. As expected, MPT was found to be induced by arachidonic acid (AA), and suppressed by CsA, BSA and L-carnitine (Fig. 5).

3.6. Arachidonic acid-induced Cyt. c release and inhibition by CsA, BSA and L-carnitine

Cyt. *c* was released during the time of MPT induced by AA, but was suppressed in the presence of CsA, BSA and L-carnitine (Fig. 6).

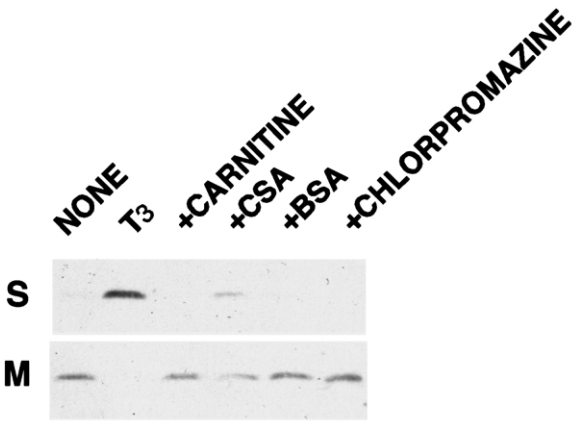

Fig. 4. Effects of various reagents on T_3-induced Cyt. *c* release. Experimental conditions were as described for Fig. 1. The figure shows Western blot analysis of Cyt. *c* during T_3-induced MPT. Mitochondria were treated with 25 μM T_3 for 10 min in the presence or absence of various reagents and fractioned into mitochondria (M) and supernatant (S). Reagents used were L-carnitine 1 mM, BSA 0.1 mg/ml, chlorpromazine 10 μM and CsA 2 μM.

4. Discussion

The tail of anuran tadpoles, comprising the epidermis, connective tissue, muscle, etc., com-

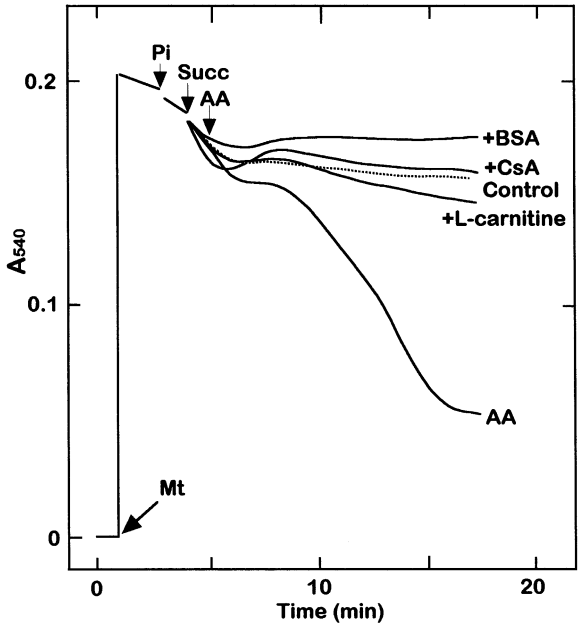

Fig. 5. Arachidonic acid-induced MPT and its inhibition by CsA, BSA and L-carnitine. Experimental conditions were as described for Fig. 1. Concentrations used were arachidonic acid (AA) 3 μM, CsA 1 μM, BSA 0.1 mg/ml, and L-carnitine 1 mM. AA induced mitochondrial swelling.

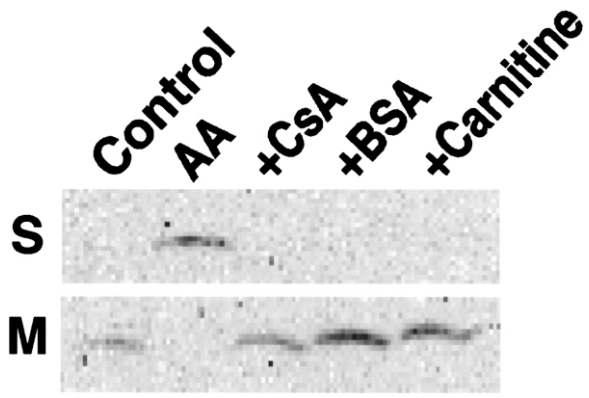

Fig. 6. Arachidonic acid-induced Cyt. *c* release and inhibition by CsA, BSA and L- carnitine. Experimental conditions were as described for Fig. 1. The figure shows Western blot analysis of Cyt. *c* during AA-induced MPT of mitochondria. Mitochondria were treated with 3 μM AA for 10 min in the presence or absence of CsA 2 μM, BSA 0.1 mg/ml or L-carnitine 1 mM, and fractionated into mitochondria (M) and supernatant (S).

pletely degenerates through apoptosis, or PCD, during metamorphosis. Amphibian metamorphosis is under the control of thyroid hormone (Dodd and Dodd, 1976), which regulates the expression of various genes (Shi, 1994), making this hormone useful for the study of PCD during ontogenetic development (Nishikawa and Yoshizato, 1986; Nishikawa et al., 1989; Brown et al., 1996; Shi, 1996). Recent studies have shown that mitochondria play an important role in apoptosis (Mignotte and Vayssiere, 1998), and Cyt. *c* released from mitochondria through MPT stimulate caspase cascade, thereby initiating apoptosis (Zoratti and Szabó, 1995; Green and Reed, 1998). It has also been demonstrated that thyroid hormone induces the release of fatty acids, which in turn induces mitochondrial swelling (Woitczak and Lehninger, 1961), with this mitochondrial swelling being prevented by BSA binding to free fatty acids. It has also been shown that fatty acids induce MPT in a CsA-inhibitable manner (Wieckowski et al., 2000). The inhibitory effect of CsA on PLA_2 activity has also been reported (Broekemeier and Pfeiffer, 1995). The release fatty acids is also thought to be induced by PLA_2 (Marzoev and Vladimirov, 1977), and fatty acid release has been shown to be inhibited in the presence of chlorpromazine, a known inhibitor of PLA_2. Trifluoperazine, a PLA_2 inhibitor, induces the same inhibition of MPT (data not shown). These findings suggest that thyroid hormone might induce the opening of MPT pore. In fact, it was found that mitochondrial MPT is induced by T_3 in vitro and in vivo (Kalderon et al., 1995; Malkevitch et al., 1997; Castilho et al., 1998). Furthermore, a recent investigation showed that thyromimetic fatty acid analogue induces mitochondrial protonophoric activity (Hermesh et al., 2000). These results further support the idea that thyroid hormone-induced MPT is correlated with the generation of free fatty acid during mitochondrial swelling.

In the present study it was found that both CsA and L-carnitine inhibit T_3-induced MPT and Cyt. *c* release in isolated mitochondria. CsA effectively suppressed T_3-induced mitochondrial MPT, which is comparable to the action of bongkrekic acid, a marker of MPT (Lê Quôc and Lê Quôc, 1988). L-carnitine facilitates the transport of LCFAs into the mitochondrial matrix, thereby enhancing β-oxidation. Because cephaloridin, a potent inhibitor of the carnitine transporter, abolished the inhibitory effect of L-carnitine, the protective effect of L-carnitine might occur in and around mitochondrial MPT pore (Furuno et al., 2001). Hence, the suppressive effects of L-carnitine on the occurrence of T_3-induced MPT and Cyt. *c* release might relate to the removal of LCFAs and the enhancement of β-oxidation that facilitates ATP formation. It is not known, however, whether the generation of fatty acids in and around the mitochondrial membrane occurs prior to the occurrence of MPT or after.

Mitochondrial PLA_2 is localized predominantly in the inner membrane, and its activity at or near the contact sites of the outer and inner membranes, the site of the MPT pore, is about two-times greater than that at other sites on the inner membranes (Levrat and Louisot, 1992). A wide variety of toxic compounds release mitochondrial Ca^{2+} by increasing the permeability of the inner membrane (Broekemeier et al., 1985). These findings suggest that free fatty acids and lysophospholipids might accumulate in the mitochondria through activation of Ca^{2+}-dependent PLA_2 prior to the induction of certain MPT's (Pfeiffer et al., 1979).

The molecular mechanism by which T_3 and LCFAs enhance mitochondrial MPT is not clear at present. The effect of LCFAs is similar to that of carboxyatractyloside, a specific inhibitor of the ADP/ATP carrier (AAC) (Schonfeld and Bohnensack, 1997). In this case, LCFAs induce MPT not only by their protonophilic action (mediated by mitochondrial anion carriers), but also by an

interaction with the proteins of the MPT pore (Wieckowski and Wojtczak, 1998). LCFA and carboxyatractyloside bind to AAC proteins with high affinity, thereby inhibiting anion-conducting channels (Halle-Smith et al., 1988; Schonfeld and Bohnensack, 1997). These observations suggest that T_3-induced MPT is partly regulated by a dynamic balance between LCFAs and L-carnitine in and around the mitochondrial membrane. The specific molecular mechanism of LCFA-induced MPT requires further clarification.

Acknowledgements

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References

Alnemri, E.S., Livingston, D.J., Nicholson, D.W. et al., 1996. Human ICE/CED-3 protease nomenclature. Cell 87, 171.

Arita, K., Kobuchi, H., Utsumi, T., Takehara, Y., Akiyama, J., Inoue, M., Utsumi, K., 2001. Mechanism of apoptosis in HL-60 cells induced by *n*-3 and *n*-6 polyunsaturated fatty acids. Biochem. Pharmacol. In press.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Broekemeier, K.M., Pfeiffer, D.R., 1995. Inhibition of the mitochondrial permeability transition by cyclosporin A during long time frame experiments: relationship between pore opening and the activity of mitochondrial phospholipases. Biochemistry 34, 16440–16449.

Broekemeier, K.M., Schmid, P.C., Schmid, H.H., Pfeiffer, D.R., 1985. Effects of phospholipase A_2 inhibitors on ruthenium red-induced Ca^{2+} release from mitochondria. J. Biol. Chem. 260, 105–113.

Brown, D.D., Wang, Z., Furlow, J.D. et al., 1996. The thyroid hormone-induced tail resorption program during *Xenopus laevis* metamorphosis. Proc. Natl. Acad. Sci. USA 93, 1924–1929.

Castilho, R.F., Kowaltowski, A.J., Vercesi, A.E., 1998. 3,5,3′-triiodothyronine induces mitochondrial permeability transition mediated by reactive oxygen species and membrane protein thiol oxidation. Arch. Biochem. Biophys. 354, 151–157.

Dodd, M.H.I., Dodd, J.M., 1976. The biology of metamorphosis. In: Lofts, B. (Ed.), Physiology of the Amphibia, vol. 3. Academic Press, New York, pp. 467–599.

Furuno, T., Kanno, T., Arita, K., Utsumi, T., Kajitani, M., Doi, Y., Inoue, M., Utsumi, K., 2001. Role of long chain fatty acids and carnitine in membrane permeability transport of mitochondria and apoptosis of HL-60 cells. Biochem. Pharmacol. In press.

Ghorbel, M., Seugnet, I., Ableitner, A.M., Hassan, A., Demeneix, B.A., 1997. T_3 treatment increases mitosis, then bax expression and apoptosis in the optic lobe of the chick embryo. Neurosci. Lett. 231, 127–130.

Green, D.R., Reed, J.C., 1998. Mitochondria and apoptosis. Science 281, 1309–1312.

Halle-Smith, S.C., Murray, A.G., Selwyn, M.J., 1988. Palmitoyl-CoA inhibits the mitochondrial inner membrane anion-conducting channel. FEBS Lett. 236, 155–158.

Hermesh, O., Kalderon, B., Berman, B., Bar-Tana, J., 2000. Mitochondrial protonophoric activity induced by a thyromimetic fatty acid analogue. Biochim. Biophys. Acta 1457, 166–174.

Hoffman, J.F., Laris, P.C., 1974. Determination of membrane potential in human and amphibia red blood cells by means of fluorescent probe. J. Physiol. 239, 519–552.

Hogeboom, G.H., 1955. Fractionation cell components and animal tissues. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods Enzymol, vol. 1. Academic Press, New York, pp. 16–19.

Kalderon, B., Hermesh, O., Bar-Tana, J., 1995. Mitochondrial permeability transition is induced by in vivo thyroid hormone treatment. Endocrinology 136, 3552–3556.

Kanno, T., Arita, K., Utsumi, T. et al., 2000. Mitochondrial membrane permeability transition and its sensitivity to L-carnitine. In: Yoshikawa, T., Toyokuni, S., Yamamoto, Y., Naito, Y. (Eds.), Free Radical in Chemistry, Biology and Medicine. OICA International, London, pp. 191–214.

Kerr, J.F.R., Harmon, B., Searle, J., 1974. An electron-microscope study of cell deletion in the anuran tadpole tail during spontaneous metamorphosis with special reference to apoptosis of striated muscle fibers. J. Cell Sci. 14, 571–585.

Lê Quôc, K., Lê Quôc, D., 1988. Involvement of the ADP/ATP carrier in calcium-induced perturbations of the mitochondrial inner membrane permeability: importance of the orientation of the nucleotide binding site. Arch. Biochem. Biophys. 265, 249–257.

Levrat, C., Louisot, P., 1992. Dual localization of the mitochondrial phopholipase A_2: outer membrane contact sites and inner membrane. Biochem. Biophys. Res. Commun. 183, 719–724.

Li, P., Nijhawan, D., Budihardjo, I. et al., 1997. Cytochrome *c* and dATP-dependent formation of

Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91, 479–489.
Little, G.H., Flores, A., 1992. Inhibition of programmed cell death by cyclosporin. Comp. Biochem. Physiol. 103C, 463–467.
Malkevitch, N.V., Dedukhova, V.I., Simonian, R.A., Skulachev, V.P., Starkov, A.A., 1997. Thyroxine induces cyclosporin A-insensitive, Ca^{2+}-dependent reversible permeability transition pore in rat liver mitochondria. FEBS Lett. 412, 173–178.
Marzoev, A.I., Vladimirov, I.A., 1977. Role of mitochondrial membrane lipid hydrolysis in their swelling induced by thyroxine. Biull. Eksp. Biol. Med. 84, 565–567.
Mignotte, B., Vayssiere, J.-L., 1998. Mitochondria and apoptosis. Eur. J. Biochem. 252, 1–15.
Mustafa, M.G., Utsumi, K., Packer, L., 1966. Damped oscillatory control of mitochondrial respiration and volume. Biochem. Biophys. Res. Commun. 24, 381–385.
Nishikawa, A., Kaiho, M., Yashizato, K., 1989. Cell death in the anuran tadpole tail: thyroid hormone induces keratinization and tail-specific growth inhibition of epidermal cells. Dev. Biol. 131, 337–344.
Nishikawa, A., Yoshizato, K., 1986. Hormonal regulation of growth and life span of bullfrog tadpole tail epidermal cells cultured in vitro. J. Exp. Zool. 237, 221–230.
Pfeiffer, D.R., Schmid, P.C., Beatrice, M.C., Schmid, H.H., 1979. Intramitochondrial phopholipase activity and the effects of Ca^{2+} plus N-ethylmaleimide on mitochondrial function. J. Biol. Chem. 254, 11485–11494.
Raff, M., 1998. Cell suicide for beginners. Nature 396, 119–122.
Reed, J.C., 1997. Cytochrome *c*: can't live with it—can't live without it. Cell 91, 559–562.
Sachs, L.M., Abdallah, B., Hassan, A. et al., 1997. Apoptosis in Xenopus tadpole tail muscles involves Bax-dependent pathways. FASEB J. 11, 801–808.
Schonfeld, P., Bohnensack, R., 1997. Fatty acid-promoted mitochondrial permeability transition by membrane depolarization and binding to the ADP/ATP carrier. FEBS Lett. 420, 167–170.
Shi, Y.-B., 1994. Molecular biology of amphibian metamorphosis. A new approach to an old problem. TEM 5, 14–20.
Shi, Y.-B., 1996. Thyroid hormone-regulated early and late genes during amphibian metamorphosis. In: Gilbert, L.I., Tata, J.R., Atkinson, B.G. (Eds.), Metamorphosis: Post-embryonic Reprogramming of Gene Expression in Amphibian and Insect Cells. Academic Press, San Diego, pp. 505–538.
Shi, Y.-B., 2000. Amphibian Metamorphosis from Morphology to Molecular Biology. Wiley-Liss, New York, Chichester, Weinheim, Brisbane, Singapore, Toronto.
Shi, Y.-B., Wong, J., Puzianowska-Kuznicka, M., Stolow, M.A., 1996. Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. BioEssays 18, 391–399.
Su, Y., Shi, Y., Shi, Y.-B., 1997a. Cyclosporin A but not FK506 inhibits thyroid hormone-induced apoptosis in tadpole intestinal epithelium. FASEB J. 11, 559–565.
Su, Y., Shi, Y., Stolow, M.A., Shi, Y.-B., 1997b. Thyroid hormone induces apoptosis in primary cell cultures of tadpole intestine: cell type specificity and effects of extracellular matrix. J. Cell Biol. 139, 1533–1543.
Sultan, A., Sokolove, P.M., 2001. Free fatty acid effects on mitochondrial permeability: an overview. Arch. Biochem. Biophys. 386, 52–61.
Tata, J.R., 1993. Gene expression during metamorphosis: an ideal model for post-embryonic development. BioEssays 15, 239–248.
Thompson, C.B., 1995. Apoptosis in the pathogenesis and treatment of disease. Science 267, 1456–1462.
Thornberry, N.A., Lazebnik, Y., 1998. Caspases: enemies within. Science 281, 1312–1316.
Utsumi, T., Okuma, M., Kanno, T. et al., 1995. Effect of the antiretroviral agent hypericin on rat liver mitochondria. Biochem. Pharmacol. 50, 655–662.
Waring, P., Beaver, J., 1996. Cyclosporin A rescues thymocytes from apoptosis induced by very low concentrations of thapsigargin: effects on mitochondrial function. Exp. Cell Res. 227, 264–276.
Wieckowski, M.R., Brdiczka, D., Wojtczak, L., 2000. Long-chain fatty acids promote opening of the reconstituted mitochondrial permeability transition pore. FEBS Lett. 484, 61–64.
Wieckowski, M.R., Wojtczak, L., 1998. Fatty acid-induced uncoupling of oxidative phosphorylation is partly due to opening of the mitochondrial permeability transition pore. FEBS Lett. 423, 339–342.
Woitczak, L., Lehninger, A.L., 1961. Formation of disappearance of an endogenous uncoupling factor during swelling and contraction of mitochondria. Biochim. Biophys. Acta 51, 442–456.
Yabuki, M., Tsutsui, K., Horton, A.A., Yoshioka, T., Utsumi, K., 2000. Caspase activation and cytochrome *c* release during HL-60 cell apoptosis induced by a nitric oxide donor. Free Radic. Res. 32, 507–514.
Yang, J.C., Cortopassi, G.A., 1998. Induction of the mitochondrial permeability transition causes release of the apoptogenic factor cytochrome *c*. Free Radic. Biol. Med. 24, 624–631.
Zoratti, M., Szabó, I., 1995. The mitochondrial permeability transition. Biochim. Biophys. Acta 1241, 139–176.