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TIANEPTINE, A NEW TRICYCLIC ANTIDEPRESSANT METABOLIZED BY β-OXIDATION OF ITS HEPTANOIC SIDE CHAIN, INHIBITS THE MITOCHONDRIAL OXIDATION OF MEDIUM AND SHORT CHAIN FATTY ACIDS IN MICE

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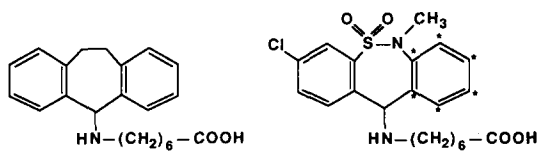
Abstract—Tianeptine is a new tricyclic antidepressant which is metabolized mainly by β-oxidation of its heptanoic side chain. We determined the effects of tianeptine on the mitochondrial oxidation of natural fatty acids in mice. *In vitro*, tianeptine (0.5 mM) inhibited by only 32% the formation of β-oxidation products from [1-^{14}C]palmitic acid by hepatic mitochondria, but inhibited by 71% that from [1-^{14}C]octanoic acid and by 51% that from [1-^{14}C]butyric acid. The activity of the tricarboxylic acid cycle, assessed as the *in vitro* formation of [^{14}C]CO_2 from [1-^{14}C]acetylcoenzyme A was decreased by 51% in the presence of tianeptine (0.5 mM). The inhibition of both β-oxidation and the tricarboxylic acid cycle appeared reversible in mitochondria from mice exposed to tianeptine *in vivo* but incubated *in vitro* without tianeptine. *In vivo*, administration of tianeptine (0.0625 mmol/kg i.p.), decreased by 53 and 58%, respectively, the formation of [^{14}C]CO_2 from [1-^{14}C]octanoic acid and [1-^{14}C]butyric acid, but did not significantly decrease that from [1-^{14}C]palmitic acid. After administration of high doses of tianeptine, however, formation of [^{14}C]CO_2 from [1-^{14}C]palmitic acid became inhibited as well, transiently after 0.25 mmol/kg and durably (>24 hr) after 0.75 mmol/kg i.p. Hepatic triglycerides were increased 24 hr after administration of 0.75 mmol/kg i.p. of tianeptine, but not after 0.25 mmol/kg i.p. Microvesicular steatosis of the liver was observed in some mice after 0.75 mmol/kg i.p., but not after 0.5 mmol/kg i.p. We conclude that tianeptine inhibits the oxidation of medium- and short-chain fatty acids in mice. Microvesicular steatosis, however, requires very large doses in mice (0.75 mmol/kg i.p., i.e. 600-times the oral dose in humans), and is therefore unlikely to occur in humans.

Tianeptine (Fig. 1) is a new tricyclic antidepressant, which has been marketed in France by Ardix Laboratories since May 1988. Its main biochemical effect is an increased serotinin uptake [1]. The chemical structure of tianeptine resembles that of amineptine (Fig. 1). Both antidepressants have a related tricyclic moiety and an identical heptanoic side chain (Fig. 1). Both are metabolized mainly by β-oxidation of this fatty chain [2, 3]. It was reported recently that amineptine inhibits the β-oxidation of natural fatty acids, and produces microvesicular steatosis of the liver in mice [4]. Mild microvesicular steatosis has been reported also in two human subjects receiving amineptine [5, 6]. Microvesicular steatosis is also observed in humans exposed to hypoglycin [7], valproic acid [8], pirprofen [9, 10] and tetracycline [11].



AMINEPTINE TIANEPTINE

Fig. 1. Chemical structure of tianeptine and amineptine. Asterisks indicate the position of the radiolabel in [^{14}C]tianeptine.

All these compounds have been shown to inhibit the mitochondrial oxidation of fatty acids [7, 10, 12, 13]. Unlike macrovacuolar steatosis, which is by itself a benign liver lesion, microvesicular steatosis, when extensive, is a severe disease, possibly associated with liver failure, coma and death [11].

Due to the similarity in the chemical structure (Fig. 1) and in the metabolism [2, 3] of amineptine and tianeptine, it appeared interesting to investigate the possible effect of tianeptine on fatty acid metabolism.

The present investigation reports the effects of tianeptine on fatty acid oxidation, hepatic triglyceride levels, and liver histology in mice.

MATERIALS AND METHODS

Animals. Male Crl:CD-1 (ICR)BR Swiss mice were purchased from Charles River (Saint-Aubin-lès-Elbeuf, France). Some mice were fed *ad lib.* with a normal diet (M25 biscuits, Extra Labo, Piètrement, Provins, France). Other mice were fasted for 48 hr before being used.

Materials and treatments. The sodium salt of tianeptine, [^{14}C]tianeptine (30 mCi/mmol), and amineptine hydrochloride were kindly provided by Institut de Recherches Internationales Servier (Neuilly, France). [1-^{14}C]Butyric acid (54 mCi/mmol), [1-^{14}C]palmitic acid (54 mCi/mmol) and [U-^{14}C]palmitic acid (928 mCi/mmol) were purchased

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from Amersham (Bucks, U.K.). [1-^{14}C]Octanoic acid (caprylic acid) (54 mCi/mmol) was purchased from New England Nuclear (Boston, MA). [1-^{14}C]Acetylcoenzyme A (48 mCi/mmol) and [1-^{14}C]acetic acid (54 mCi/mmol) were purchased from Centre d'Etudes Nucléaires de Saclay (Gif-sur-Yvette, France).

The sodium salt of tianeptine was dissolved in water, followed by the addition of HCl to bring the pH back to 7.4. Mice received 0.5 ml i.p. of this tianeptine solution (0.016–0.75 mmol/kg i.p.).

[U-^{14}C]Palmitic acid (3.7 μCi/kg; 4 nmol/kg), [1-^{14}C]palmitic acid (15 μCi/kg, 274 nmol/kg), [1-^{14}C]ocatanoic acid (4 μCi/kg; 69 nmol/kg) or [1-^{14}C]butyric acid (19 μCi/kg; 343 nmol/kg) was administered by gastric intubation in 0.2 ml of corn oil.

In vitro *mitochondrial β-oxidation.* Liver mitochondria were prepared as described by McGarry *et al.* [14]. An aliquot was used to determine mitochondrial protein by the method of Lowry *et al.* [15]. The β-oxidation of [U-^{14}C]palmitic acid and [1-^{14}C]palmitic acid by liver mitochondria was assessed as described by Otto and Ontko [16] and Fréneaux *et al.* [13]. The preincubation medium (1.8 ml of 70 mM sucrose, 43 mM KCl, 3.6 mM $MgCl_2$, 7.2 mM potassium phosphate, 36 mM Tris–HCl buffer, pH 7.4) contained 0.2 mM adenosine triphosphate, 50 μM DL-carnitine, 15 μM coenzyme A and mitochondria from 100 mg of liver (about 2 mg of mitochondrial protein), with or without tianeptine (0.0625–2 mM). After 5 min of preincubation at 30°, the incubation mixture was brought to 2 ml by adding 200 μl of the same buffer containing [U-^{14}C]palmitic acid (final concentration, 40 μM; 0.05 μCi/2 ml) or [1-^{14}C]palmitic acid (final concentration, 40 μM; 0.5 μCi/2 ml) with bovine serum albumin (final concentration, 0.5 mg/2 ml). In some tubes, 2 mM KCN were added to inhibit mitochondrial β-oxidation.

The tubes were then incubated, with slow shaking, at 30°. After 10 min, the reaction was stopped by adding 0.4 ml of 5% perchloric acid into the incubation mixture. The incubation mixture was then centrifuged at 4000 *g* for 10 min and [^{14}C]acid-soluble β-oxidation products were counted in the supernatant. Such products mainly represent ketone bodies and, to a small extent, citric acid cycle intermediates [17–19]. We verified, from measurement of the KCN-inhibitable activity, that mitochondria mediated at least 70% of the oxidation of [1-^{14}C]palmitate and at least 80% of that of [U-^{14}C]palmitate. In other tubes, the concentration of albumin in the incubate was 5 mg/2 ml instead of 0.5 mg/2 ml.

The formation of β-oxidation products from [1-^{14}C]octanoic acid or [1-^{14}C]butyric acid (final concentrations, 40 μM; 0.1 μCi/2 ml) was measured after an identical preincubation and incubation protocol. However, specific extraction steps had to be added after addition of perchloric acid and centrifugation, because both octanoic acid and butyric acid are soluble in the acidic supernatant fraction.

After incubation with [1-^{14}C]octanoic acid, the supernatant (1.5 ml) was extracted twice with 5 ml of *n*-hexane. We verified that this extraction removed essentially all (99%) of [1-^{14}C]octanoic acid, while β-hydroxybutyrate and acetoacetate were not extracted.

After incubation with [1-^{14}C]butyric acid, the unextracted acid aqueous phase was first counted for ^{14}C activity. Another aliquot (1.5 ml) was then extracted five times with 4 ml of benzene, before being counted. This procedure did not extract β-hydroxybutyrate or acetoacetate, and extracted 82% of [1-^{14}C]butyric acid. Using this extraction ratio, and the difference in counts, we could calculate the remaining [1-^{14}C]butyric acid, and then [^{14}C]β-oxidation products.

In preliminary experiments, we also measured the formation of [^{14}C]CO_2 from [U-^{14}C]palmitic acid, [1-^{14}C]palmitic acid, [1-^{14}C]octanoic acid or [1-^{14}C]butyric acid. The incubation tubes were tightly capped with plastic stoppers pierced by two needles and were continuously swept by an air flow which was bubbled into 14 ml of ethanolamine-2-methoxyethanol (30/70%, v/v). Trapping of [^{14}C]CO_2 was continued for 60 min after the incubation. A 3-ml aliquot of ethanolamine-2-methoxyethanol was then counted for [^{14}C]CO_2 activity. With all the fatty acids studied, the formation of [^{14}C]CO_2 remained negligible (about 5% of the radioactivity associated with total oxidized products) and was therefore not measured afterwards.

In vitro *formation of* [^{14}C]CO_2 *from* [1-^{14}C]*acetylcoenzyme A or* [1-^{14}C] *acetate.* The activity of the tricarboxylic acid cycle was assessed as previously described [4, 13]. The preincubation medium (1.8 ml of 70 mM sucrose, 43 mM KCl, 3.6 mM $MgCl_2$, 7.2 mM potassium phosphate, 36 mM Tris–HCl buffer, pH 7.4) contained mitochondria from 100 mg of liver or from 200 mg of kidneys, and either nothing else or tianeptine (0.0625–2 mM). After 5 min of preincubation at 30°, the incubation medium was brought to 2 ml by adding 200 μl of the same buffer with [1-^{14}C]acetylcoenzyme A to give a final concentration of 40 μM (0.06 μCi/2 ml). The tubes were then tightly capped with a plastic stopper pierced by two needles, and were continuously swept by an air flow which was bubbled into 14 ml of ethanolamine-2-methoxyethanol (30/70%, v/v). After 10 min of incubation at 30°, the reaction was stopped by injecting 0.4 ml of 5% perchloric acid into the incubation mixture. Trapping of [^{14}C]CO_2 was continued for 60 min. A 3-ml aliquot of ethanolamine-2-methoxyethanol was then counted for [^{14}C]CO_2 activity.

In other flasks, the assay was performed exactly as described above, but with [1-^{14}C]acetate (40 μM, 0.22 μCi/2 ml) instead of [1-^{14}C]acetylcoenzyme A.

In vivo *formation of* [^{14}C]CO_2 *from* [^{14}C]*fatty acids.* A tracer dose of [U-^{14}C]palmitic acid (3.7 μCi/kg; 4 nmol/kg) was administered by gastric intubation, in 0.2 ml of corn oil, at various times (15 min–24 hr) after the administration of various doses of tianeptine (0.016–0.75 mmol/kg i.p.), in mice fasted for 48 hr. The animal was then placed for 3 hr in a small plastic cage swept by an air flow of 50 ml/min. The outflow was bubbled into 50 ml of an ethanolamine-2-methoxyethanol mixture (30/70%, v/v). After 3 hr, 3 ml were removed and counted for [^{14}C]CO_2 activity.

In other experiments, exhalation of [^{14}C]CO_2 was measured after intragastric administration of [1-

^{14}C]palmitic acid (15 μCi/kg; 274 nmol/kg), [1-^{14}C]octanoic acid (4 μCi/kg; 69 nmol/kg) or [1-^{14}C]butyric acid (19 μCi/kg; 343 nmol/kg) to mice fasted for 48 hr. Exhalation was measured for 3 hr after [1-^{14}C]palmitic acid, 15 min after [1-^{14}C]octanoic acid and 30 min after [1-^{14}C]butyric acid, i.e. the respective time periods during which exhalation remained linear with time after administration of these fatty acids.

Time course for total drug concentrations. Fed mice received [^{14}C]tianeptine (0.5 mmol/kg i.p.; 100 μCi/kg) and were killed 1, 12 or 24 hr later. A blood sample was taken. The liver and the kidneys were removed and homogenized in 3 vol. 0.15 M NaCl. Aliquots were counted for [^{14}C]tianeptine and [^{14}C]metabolites.

Plasma β-hydroxybutyrate, plasma aceoacetate and blood glucose. Plasma β-hydroxybutyrate and acetoacetate concentrations were measured by the technique of Williamson *et al.* [20], as modified by McGarry *et al.* [21]. Blood glucose concentrations were determined using a commercial kit (Sigma Kit no. 510) based on the glucose oxidase method.

Hepatic lipids and liver histology. Hepatic lipids and triglycerides were measured as previously described by Genève *et al.* [10]. In some mice, liver fragments were sampled, cut with a cryostat and stained with Oil Red 0.

Statistical analysis. Student's *t*-test for independent data or Dunnett's test were used to assess the significance of differences between means, as appropriate.

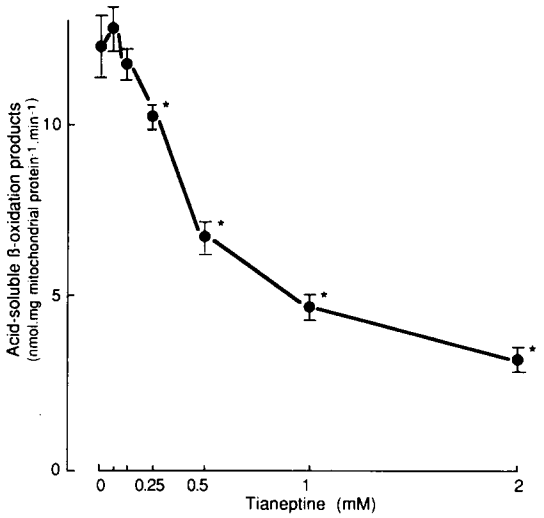

Fig. 2. Effects of various concentrations of tianeptine on the *in vitro* formation of [^{14}C]acid-soluble β-oxidation products from [U-^{14}C]palmitic acid. Mouse liver mitochondria were incubated at 30° for 10 min with [U-^{14}C]palmitic acid (40 μM, 0.05 μCi/2 ml), ATP, carnitine and coenzyme A in the presence of various concentrations (0.0625–2 mM) of tianeptine. Results are means ± SE for 4–10 experiments. Asterisks indicate significant differences from values in concomitant experiments made without tianeptine ($P < 0.05$).

RESULTS

In vitro *mitochondrial β-oxidation*

β-Oxidation was first assessed by the formation of [^{14}C]acid-soluble β-oxidation products during incubation of mouse liver mitochondria with [U-^{14}C]palmitic acid, ATP, carnitine and coenzyme A. Formation of acid-soluble β-oxidation products from [U-^{14}C]palmitic acid was linear with time for at least 10 min (not shown). Therefore all further incubations were brought to an end after 10 min. The formation of [^{14}C]acid-soluble β-oxidation products from [U-^{14}C]palmitic acid by mouse liver mitochondria was decreased by 74, 62, 45 and 16%, respectively, in the presence of 2, 1, 0.5 and 0.25 mM of tianeptine (Fig. 2).

Since mitochondrial fractions are contaminated with peroxisomes, two additional experiments were performed. In a first assay, the albumin concentration was increased from 0.5 mg/2 ml to 5 mg/2 ml. This is known to increase the fraction of the activity that is mediated by mitochondria [22]. In this modified assay, the β-oxidation of [U-^{14}C]palmitic acid was decreased by 92, 87 and 71% in the presence of 2, 1 and 0.5 mM tianeptine, respectively. In a second series of assays, performed with 0.5 mg albumin/2 ml, the mitochondrial activity was assessed as the difference between the activity measured in the absence of KCN and that in the presence of 2 mM KCN [22]. This KCN-inhibitable, mitochondrial, activity was inhibited by 90% in the presence of 1 mM tianeptine, from 9.4 ± 0.2 nmol/mg protein/min to 0.9 ± 0.1 (mean ± SE for 3 determinations). The KCN-insensitive, presumably peroxisomal, activity was only 2.1 ± 0.1 nmol/mg protein/min both in the absence of tianeptine, and in the presence of 1 mM tianeptine, suggesting that tianeptine does not inhibit this fraction.

Reversibility of the inhibitory effects of tianeptine on the β-oxidation of [U-^{14}C]palmitic acid was tested by taking advantage of the fact that the preparation of liver mitochondria involves several dilutions and washings [14]. Therefore, most of the tianeptine initially present in the livers of tianeptine-treated mice may be removed during these procedures, and little may persist in isolated mitochondria. Indeed, the *in vitro* formation of [^{14}C]acid-soluble β-oxidation products (mean ± SE for 4 experiments) was similar with mitochondria from control mice (8.8 ± 0.3 nmol/mg protein/min) and mitochondria from mice killed 4 hr after the administration of 0.75 mmol/kg i.p. of tianeptine (8.8 ± 0.1 nmol/mg protein/min).

The β-oxidation of [U-^{14}C]palmitic acid determines the oxidation of the whole chain length. It was therefore of interest to see whether inhibition affected to the same extent the oxidation of long-, medium-, or short-chain fatty acids labelled only on the carboxylic group. Tianeptine (0.5 mM) slightly decreased the *in vitro* mitochondrial β-oxidation of [1-^{14}C]palmitic acid, whereas it markedly decreased that of [1-^{14}C]octanoic acid and butyric acid (Fig. 3).

In vitro *formation of* [^{14}C]CO_2 *from* [1-^{14}C]*acetylcoenzyme A or* [1-^{14}C]*acetate*

The activity of the tricarboxylic acid cycle was

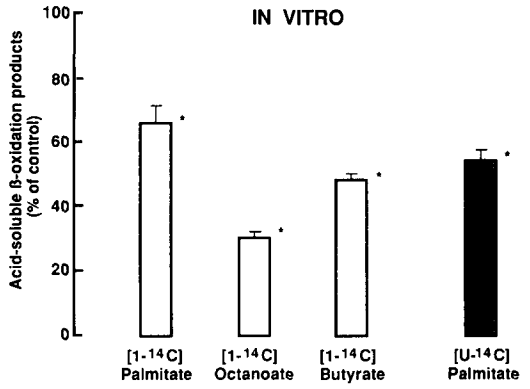

Fig. 3. Differential effects of tianeptine (0.5 mM) on the *in vitro* formation of [^{14}C]acid-soluble β-oxidation products from [1-^{14}C]fatty acids of various chain lengths. Mouse liver mitochondria were incubated at 30° for 10 min with [1-^{14}C]palmitic acid (40 μM, 0.5 μCi/2 ml), [1-^{14}C]octanoic acid (40 μM, 0.1 μCi/2 ml) or [1-^{14}C]butyric acid (40 μM, 0.1 μCi/2 ml), and with ATP, carnitine and coenzyme A. Values in the presence of tianeptine are expressed as per cent of values in the absence of tianeptine. Control values were 0.76 ± 0.06, 3.08 ± 0.11 and 1.20 ± 0.09 nmol β-oxidation products/mg mitochondrial protein/min with [1-^{14}C]palmitic acid, [1-^{14}C]octanoic acid, and [1-^{14}C]butyric acid, respectively. Results are means ± SE for 10–14 determinations. For comparison, the formation of [^{14}C]acid-soluble β-oxidation products from [U-^{14}C]palmitic acid is also shown. Asterisks indicate significant differences between means ($P < 0.01$.)

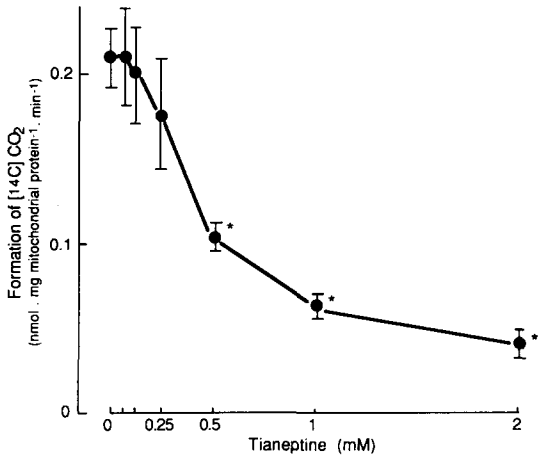

Fig. 4. Effects of various concentrations of tianeptine in the *in vitro* formation of [^{14}C]CO_2 from [1-^{14}C]acetylcoenzyme A. Mouse liver mitochondria were incubated at 30° for 10 min with [1-^{14}C]acetylcoenzyme A (40 μM, 0.06 μCi/2 ml), in the presence of various concentrations of tianeptine (0.0625–2 mM). Results are means ± SE for 4–12 experiments. Asterisks indicate significant differences from values in concomitant experiments made without tianeptine ($P < 0.05$).

first assessed by the formation of [^{14}C]CO_2 during incubation of mouse liver or kidney mitochondria with [1-^{14}C]acetylcoenzyme A. Formation of [^{14}C]CO_2 by liver mitochondria was inhibited by 81, 70 and 51%, respectively, in the presence of 2, 1 and 0.5 mM of tianeptine (Fig. 4). Formation of [^{14}C]CO_2 from [1-^{14}C]acetate was also decreased, from 0.115 ± 0.020 nmol/mg protein/min in control incubations, to 0.055 ± 0.024 in the presence of 1 mM tianeptine (mean ± SE for 6 determinations).

In vitro formation of [^{14}C]CO_2 from [1-^{14}C]acetylcoenzyme A (mean ± SE for 7–8 experiments) was not significantly different with liver mitochondria from control mice (0.093 ± 0.022 nmol/mg protein/min) and with liver mitochondria from mice killed 4 hr after the administration of 0.75 mmol/kg i.p. of tianeptine (0.073 ± 0.009 nmol/mg protein/min).

Formation of [^{14}C]CO_2 by kidney mitochondria was also inhibited markedly by tianeptine, from 0.026 ± 0.002 nmol [^{14}C]CO_2/mg protein/min in control incubations, to 0.008 ± 0.004 in the presence of 2 mM tianeptine (mean ± SE for 7 and 8 determinations).

In vivo *formation of* [^{14}C]CO_2 *from* [^{14}C] *fatty acids*

Experiments were performed in mice fasted for 48 hr and kept fasted throughout the experiment. In a first series of experiments, we determined the time course for the inhibition of the exhalation of [^{14}C]CO_2 from [U-^{14}C]palmitic acid at various times after the administration of a constant dose of tianeptine (0.75 mmol/kg i.p.). Cumulative exhalation of [^{14}C]CO_2 for the next 3 hr was decreased by 76, 84, 79 and 54%, respectively, in mice receiving [U-^{14}C]palmitic acid 15 min, 8 hr, 16 hr and 24 hr after the administration of tianeptine (not shown).

In a second series of experiments, we determined the effects of various doses of tianeptine (0.016–0.75 mmol/kg i.p.) given 15 min before [U-^{14}C]palmitic acid (Fig. 5). The cumulative exhalation of [^{14}C]CO_2 for the next 3 hr was decreased by 76, 72, 72, 61, 51 and 28%, respectively, after administration of 0.75, 0.5, 0.25, 0.125, 0.0625 and 0.031 mmol/kg of tianeptine (Fig. 5); it was unchanged after administration of 0.016 mmol/kg of tianeptine (Fig. 5).

In a third series of experiments we determined the effects of tianeptine (0.0625 mmol/kg i.p. given 15 min before the fatty acid) on the formation of [^{14}C]CO_2 from [1-^{14}C]fatty acids of various chain lengths. Formation of [^{14}C]CO_2 from [1-^{14}C]palmitic acid was not significantly inhibited, whereas formation of [^{14}C]CO_2 from [1-^{14}C]octanoic and [1-^{14}C]butyric acid were decreased by 53 and 58%, respectively (Fig. 6).

In a fourth series of experiments, we determined the time course for the inhibition of the exhalation of [^{14}C]CO_2 from [1-^{14}C]palmitic acid, administered 15 min or 24 hr after the administration of tianeptine (0.25 or 0.75 mmol/kg). The cumulative exhalation of [^{14}C]CO_2 for the next 3 hr was decreased by 77 and 82%, respectively, 15 min after administration of 0.25 and 0.75 mmol/kg of tianeptine; 24 hr after administration of tianeptine, however, the cumulative exhalation of [^{14}C]CO_2 was no longer significantly decreased after 0.25 mmol/kg of tianeptine whereas it was still decreased by 61% after 0.75 mmol/kg of tianeptine (Fig. 7).

In a last series of experiments, we determined the effects of amineptine (0.25 mmol/kg i.p. given 15 min before the fatty acid) on the formation of

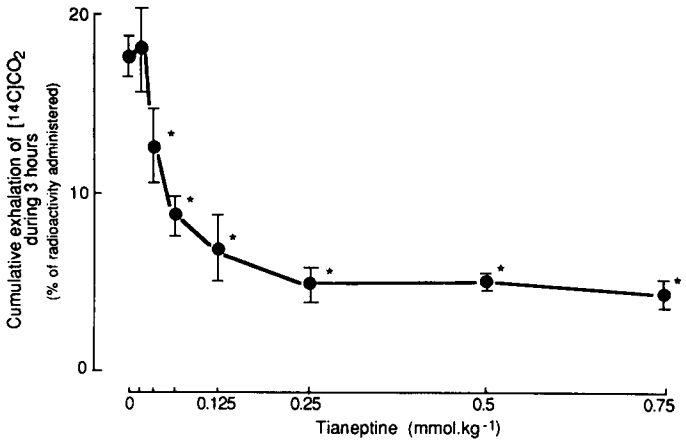

Fig. 5. Effects of various doses of tianeptine, given 15 min before [U-^{14}C]palmitic acid, on the *in vivo* exhalation of [^{14}C]CO_2. Mice fasted for 48 hr received various doses of tianeptine (0.016–0.75 mmol/kg i.p.), 15 min before the administration of a tracer dose of [U-^{14}C]palmitic acid (3.7 μCi/kg) given by gastric intubation. The exhalation of [^{14}C]CO_2 was measured during the next 3 hr. Results are means ± SE for 5–12 mice. Asterisks indicate significant differences from values in control mice ($P < 0.05$).

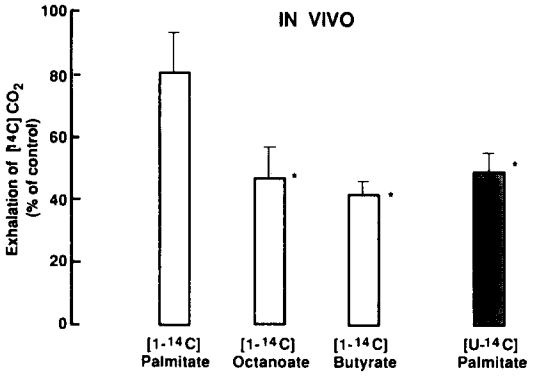

Fig. 6. Differential effects of tianeptine (0.0625 mmol/kg i.p.), given 15 min before the labelled fatty acid, on the *in vivo* formation of [^{14}C]CO_2 from [1-^{14}C]fatty acids of various chain lengths. Mice fasted for 48 hr received tianeptine 15 min before a tracer dose of [1-^{14}C]palmitic acid (15 μCi/kg), [1-^{14}C]octanoic acid (4 μCi/kg) or [1-^{14}C]butyric acid (19 μCi/kg) given by gastric intubation. Exhalation of [^{14}C]CO_2 was measured for 3 hr after [1-^{14}C]palmitic acid, 15 min after [1-^{14}C]octanoic acid and 30 min after [1-^{14}C]butyric acid. Values in mice treated with tianeptine are expressed as per cent of values in control mice. Control values were 42 ± 5, 30 ± 2 and 46 ± 5% of the radioactivity administered with [1-^{14}C]palmitate, [1-^{14}C]octanoate and [1-^{14}C]butyrate, respectively. Results are means ± SE for 6 mice. For comparison, the formation of [^{14}C]CO_2 from [U-^{14}C]palmitic acid is also shown. Asterisks indicate significant differences between means ($P < 0.01$).

[^{14}C]CO_2 from [1-^{14}C]fatty acids of various chain lengths. Oxidation of [1-^{14}C]palmitic acid was inhibited by only 35%, whereas oxidation of [1-^{14}C]octanoic acid and [1-^{14}C]butyric acid was inhibited by 67 and 68%, respectively (data not shown).

Time course for total drug concentrations

The total concentration of [^{14}C]tianeptine and [^{14}C]metabolites 1, 12 and 24 hr after the administration of [^{14}C]tianeptine (0.5 mmol/kg i.p.) was, respectively, 0.51 ± 0.03, 0.056 ± 0.005 and 0.016 ± 0.001 μmol/ml in blood, 1.05 ± 0.03, 0.22 ± 0.04 and 0.024 ± 0.002 μmol/g in liver, and 0.89 ± 0.08, 0.127 ± 0.012 and 0.019 ± 0.002 μmol/g in kidney (mean ± SE for 6 mice).

Plasma ketone bodies

Plasma ketone bodies were determined after the administration of 0.5 mmol/kg of tianeptine in mice fasted for 48 hr. Six hours after tianeptine, plasma D(−)-β-hydroxybutyrate was not significantly increased, while acetoacetate was significantly increased (Table 1). Accordingly, the D(−)-β-hydroxybutyrate/acetoacetate ratio was decreased from 3.52 in control mice to 2.35 in tianeptine-treated mice (Table 1).

At later times, there was a marked increase in plasma ketone bodies, plasma D(−)-β-hydroxybutyrate being increased 2.3-fold and 2.8-fold, respectively, 10 and 16 hr after the administration of tianeptine, as compared to values in control mice (not shown). Plasma D(−)-β-hydroxybutyrate had returned to normal by 24 hr.

Blood glucose

Blood glucose concentration was determined in mice fasted for 48 hr. Blood glucose was decreased by 40, 51, 54 and 51% respectively 1, 2, 4 and 6 hr after the i.p. administration of 0.5 mmol/kg of tianeptine. Blood glucose showed a rebound at 9 hr and had returned to normal by 12 hr (Fig. 8).

Blood glucose concentration was significantly decreased by 34% ($P < 0.01$) 2 hr after administration of 0.25 mmol/kg i.p. of tianeptine, but was unchanged 2 hr after administration of 0.125 mmol/kg of tianeptine (not shown).

Hepatic lipids and liver histology

Hepatic lipids and liver histology were determined in fed mice. Hepatic triglycerides were significantly

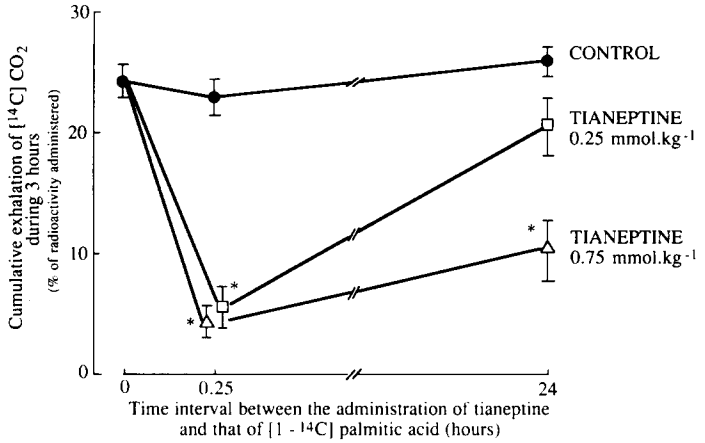

Fig. 7. Time course for the inhibition of the *in vivo* exhalation of $[^{14}C]CO_2$ from [1-^{14}C]palmitic acid after 2 different doses of tianeptine. Mice fasted for 48 hr received a tracer dose of [1-^{14}C]palmitic acid (15 μCi/kg) by gastric intubation 15 min or 24 hr after the administration of 0.25 or 0.75 mmol/kg i.p. of tianeptine. The exhalation of $[^{14}C]CO_2$ was measured during the next 3 hr. Results are means ± SE for 5–12 mice. Asterisks indicate significant differences from values in control mice ($P < 0.001$).

Table 1. Plasma D(−)-β-hydroxybutyrate and acetoacetate concentrations 6 hr after administration of tianeptine (0.5 mmol/kg i.p.)

	D(−)-β-Hydroxybutyrate (mM)	Acetoacetate (mM)	D(−)-β-Hydroxybutyrate / Acetoacetate
Control	1.2 ± 0.1	0.34 ± 0.02	3.5 ± 0.2
Tianeptine	1.4 ± 0.2	0.57 ± 0.05*	2.3 ± 0.2*

Results are means ± SE for 10 mice.
* Significantly different from that in control mice, $P < 0.01$.

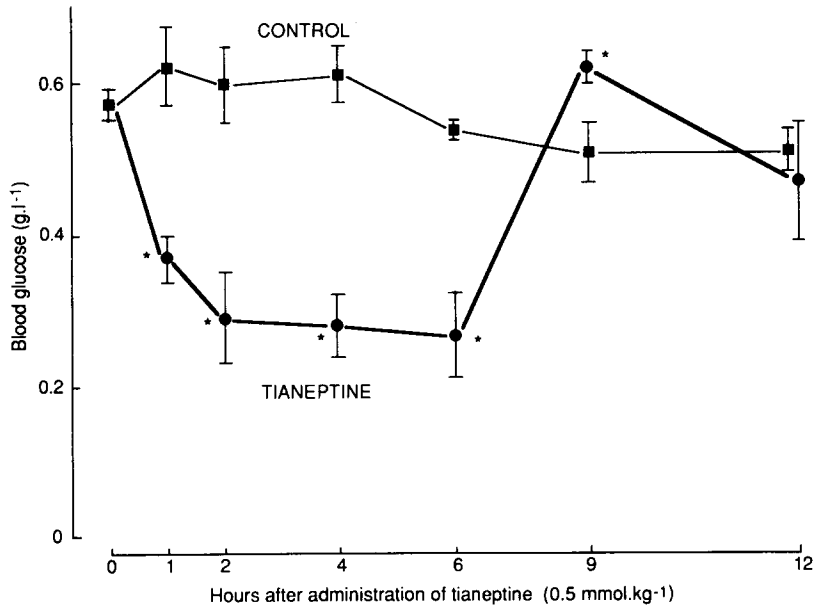

Fig. 8. Time course for blood glucose concentration after administration of tianeptine (0.5 mmol/kg i.p.) in mice fasted for 48 hr. Results are means ± SE for 4–5 mice. Asterisks indicate significant differences from control values ($P < 0.05$).

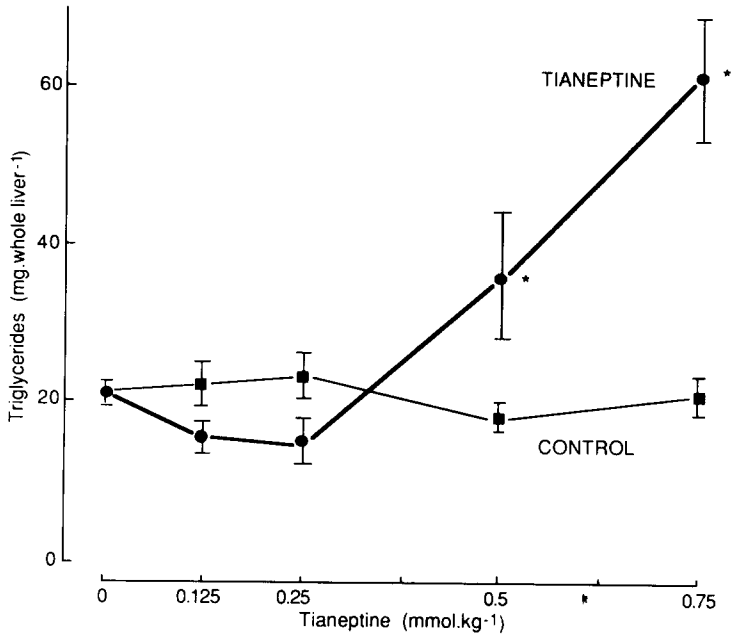

Fig. 9. Effects of various doses of tianeptine on hepatic triglycerides measured 24 hr later in fed mice. Results are means ± SE for 4–10 mice. Asterisks indicate significant difference from concomitantly killed control mice ($P < 0.05$).

increased 24 hr after administration of 0.5 or 0.75 mmol/kg i.p. of tianeptine, but not after 0.25 mmol/kg (Fig. 9). The time-course for the accumulation of hepatic triglycerides was studied after administration of 0.5 mmol/kg i.p. of tianeptine. Hepatic triglycerides were increased by 74 and 98%, respectively, 6 and 24 hr after the drug administration but had returned to normal by 48 hr (not shown).

Liver histology showed microvesicular steatosis of the liver in only 4 of 10 fed mice killed 24 hr after administration of 0.75 mmol/kg i.p. of tianeptine. Steatosis was absent in 5 mice killed 24 hr after administration of 0.5 mmol/kg i.p. of tianeptine.

DISCUSSION

Our results show that high concentrations or doses of tianeptine inhibit the oxidation of fatty acids *in vitro* (Figs 2–4) and *in vivo* (Figs 5–7), leading to the accumulation of hepatic triglycerides (Fig. 9) and the development of microvesicular steatosis of the liver in some mice. Inhibition affected both the β-oxidation process (Figs 2 and 3) and the tricarboxylic acid cycle (Fig. 4). Both effects were apparently reversible, disappearing in mitochondria first exposed to tianeptine *in vivo*, but then incubated *in vitro* without tianeptine (see Results). Tianeptine is metabolized mainly by β-oxidation of its fatty acid side chain [3]. It is therefore tempting to speculate some relationship between this metabolism and the reversible inhibition of the β-oxidation of natural fatty acids. It is noteworthy that tianeptine itself, and its two main β-oxidation metabolites, have side chains of 7, 5 and 3 carbons, respectively, making them similar in structure to medium- and short-chain natural fatty acids. It is therefore noticeable that, both *in vitro* and *in vivo*, tianeptine inhibited mainly the oxidation of medium- and short-chain fatty acids (Figs 3 and 6). Similarly, amineptine, which has an identical heptanoic side chain, and likewise forms C5 and C3 β-oxidation products [2], also inhibited mainly the oxidation of medium- and short-chain fatty acids *in vivo* (see Results). Predominant inhibition of medium- and short-chain fatty acids allows further comments on the mechanism for inhibition. Indeed, activation by coenzyme A is a prerequisite for the β-oxidation of all fatty acids, whatever their chain length [23]. In contrast, carnitine and carnitine palmitoyltransferases I and II are required for the entry into the mitochondria of long-chain fatty acids only [23]. Therefore, predominant inhibition of the β-oxidation of medium- and short-chain fatty acids by tianeptine suggests that the main mechanism for inhibition is not a depletion of either coenzyme A or carnitine, nor an inhibition of carnitine palmitoyltransferases. Taken together, these observations suggest that, at least at low doses, tianeptine mainly acts after the activation of the fatty acid and its entry into the mitochondria, by reversibly inhibiting one or several of the enzymes involved in the β-oxidation of both medium- and short-chain fatty acids.

Decreased β-oxidation of natural fatty acids may decrease the formation of NADH within the mitochondria, leading to a decreased NADH/NAD^+ ratio [24, 25]. This decreased ratio may explain the decreased plasma D(−)-β-hydroxybutyrate/acetoacetate ratio observed in the present study (Table 1), or after administration of valproic acid [12], hypoglycin [24], or pent-4-enoic acid [25]. Another effect of tianeptine was to increase total plasma ketone bodies (Table 1 and Results). Of seven compounds

shown to inhibit β-oxidation of fatty acids, only two drugs, namely pirprofen and valproic acid decreased plasma ketone bodies [10, 12], whereas the five other compounds, namely tianeptine (Table 1 and Results), amineptine [4], pent-4-enoic acid [25], hypoglycin [24] and tetracycline [13] increased, in contrast, plasma ketone bodies. It is noteworthy that amineptine [4], tetracycline [13] and tianeptine (Fig. 4) inhibited the tricarboxylic acid cycle. Inhibition by tianeptine affected not only hepatic mitochondria (Fig. 4) but also kidney mitochondria (Results). As a hypothesis, it is therefore tempting to speculate that tianeptine decreases the overall peripheral utilization of ketone bodies even more than it decreases their hepatic formation rate, explaining the increase in total plasma ketone bodies. Similar explanations have been proposed in the cases of pent-4-enoic acid [18], hypoglycin [24], amineptine [4] and tetracycline [13]. It is noteworthy that ibuprofen, which inhibits β-oxidation without inhibiting the tricarboxylic acid cycle, instead decreases total plasma ketone bodies (Fréneaux *et al.* in preparation).

Yet another effect of tianeptine was to decrease blood glucose concentrations (Fig. 8). A similar effect is observed after administration of amineptine [4], hypoglycin [7], pent-4-enoic acid [25], valproic acid [12] and pirprofen [10]. This effect has been attributed mainly to decreased hepatic gluconeogenesis [7, 18].

Although tianeptine in low doses inhibited predominantly the oxidation of medium- and short-chain fatty acids (Fig. 6), at high doses (0.25 mmol/kg and more), it also inhibited the *in vivo* oxidation of [1-^{14}C]palmitic acid (Fig. 7). Hepatic triglycerides are mostly made up of long chain fatty acids. It is therefore noteworthy that only those doses of tianeptine which were high enough to decrease durably the oxidation of long chain fatty acids, led to significant accumulation of hepatic triglycerides. Requirement for prolonged inhibition is suggested by the following observations. Administration of 0.25 mmol/kg of tianeptine decreased markedly the *in vivo* oxidation of [1-^{14}C]palmitic acid measured 15 to 195 min later, but not 24 to 27 hr later (Fig. 7). This dose of tianeptine did not increase hepatic triglycerides at 24 hr (Fig. 9). In contrast, a dose of 0.75 mmol/kg of tianeptine decreased durably the *in vivo* oxidation of [1-^{14}C]palmitic acid, even when measured 24 to 27 hr later (Fig. 7). This dose of tianeptine increased hepatic triglycerides at 24 hr (Fig. 9). Even so, the increase in hepatic triglycerides was moderate. Histologically, microvesicular steatosis was detected in some mice only (Results).

The dose of tianeptine which was required to significantly inhibit the *in vivo* oxidation of [U-^{14}C]palmitic acid (which measures the oxidation of the whole chain length) was 0.031 mmol/kg i.p. or 14 mg/kg i.p. in mice (Fig. 5). This dose is, however, 25-fold the human therapeutic dose (37.5 mg daily p.o., or about 0.55 mg/kg). The dose of tianeptine required to produce microvesicular steatosis of the liver in mice (i.e. 0.75 mmol/kg i.p. or 344 mg/kg) was 600-fold the human dose. From this enormous safety margin, it seems likely that treatments with tianeptine will not produce microvesicular steatosis of the liver in man. Indeed, up to now, this antidepressant has not resulted in any liver disease in humans [24]. With amineptine, in contrast, the safety margin for mild microvesicular steatosis was much less (10-fold), and 2 patients have been reported with mild microvesicular steatosis after administration of amineptine [5, 6].

In summary, we conclude that tianeptine (a tricyclic antidepressant metabolized by β-oxidation of its heptanoic side chain) inhibits the mitochondrial oxidation of natural fatty acids in mice. Inhibition appears reversible. It affects mainly enzymes involved in the β-oxidation of medium- and short-chain fatty acids. At very high doses (0.75 mmol/kg), the oxidation of long chain fatty acids becomes durably affected as well, and mild microvesicular steatosis is observed in some mice. This dose, however, is 600-fold the therapeutic dose, suggesting that microvesicular steatosis will probably not occur in man.

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