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Characterization and regulation of the 3β-hydroxysteroid dehydrogenase isomerase enzyme in the rat sciatic nerve

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Abstract

In the peripheral nervous system, progesterone (PROG) has a stimulatory effect on myelination. It could be derived from local synthesis, as Schwann cells in culture express the 3β-hydroxysteroid dehydrogenase (3β-HSD) and convert pregnenolone (PREG) to PROG. Although 3β-HSD mRNA can be detected by RT-PCR in peripheral nerves, the activity of the enzyme has so far not been demonstrated and characterized in nerve tissue. In this study, we show that homogenates prepared from rat sciatic nerves contain a functional 3β-HSD enzyme and we have analysed its kinetic properties and its regulation by steroids. The activity of 3β-HSD in homogenates was evaluated using ^{3}H-labelled PREG as a substrate and NAD^+ as a cofactor, the levels of steroids formed were calculated either by extrapolating the relationship between tritiated peaks obtained by TLC to the initial amount of PREG, or by gas chromatography/mass spectrometry determination. A rapid increase in PROG formation was found between 0 and 50 min of incubation and no further significant changes were observed between 1 and 4 h. The calculated K_m value (1.06 ± 0.19 μM) was close to the values described for the 3β-HSD type-I and type-IV isoforms. Trilostane, a competitive inhibitor of the 3β-HSD caused a potent inhibition of the rate of conversion of PREG to PROG (IC_{50} = 4.06 ± 2.58 μM). When the effects of different steroids were tested, both oestradiol and PROG significantly inhibited the conversion of PREG to PROG.

Keywords: neurosteroids, pregnenolone, progesterone, steroids, trilostane, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD).

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Steroids are qualified as 'neurosteroids' if they are synthesized within the nervous system (Baulieu 1991; Robel *et al*. 1999). However, in most studies, neurosteroid synthesis has only been demonstrated in cultured cells. Thus, rat Schwann cells and sensory neurons isolated, respectively, from neonatal sciatic nerves or from embryonic dorsal root ganglia, synthesize progesterone (PROG) from pregnenolone (PREG) (Guennoun *et al*. 1997; Robert *et al*. 2001). In contrast to sensory neurons, which express a functional 3β-hydroxysteroid dehydrogenase (3β-HSD) when cultured alone, Schwann cells only convert PREG to PROG after having been exposed to a diffusible neuronal factor.

In the peripheral nervous system, PROG plays a role in myelination. This has been demonstrated both in the regenerating and ageing rodent sciatic nerve and in dorsal root ganglia explant cultures (Koenig *et al*. 1995; Chan *et al*. 1998; Melcangi *et al*. 1998; Notterpek *et al*. 1999). Because PROG has such an important role in the peripheral nervous system, its has become essential to demonstrate and characterize its synthesis in nerve tissue. The synthesis of PROG involves the oxidation and isomerization of the 3β-hydroxy-Δ5-pregnene into 3-keto-Δ4-pregnene. This key enzymatic step is catalysed by isoenzymes of the 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD) family, which can transform PREG into PROG, 17-hydroxy-PREG into 17-hydroxy-PROG, dehydroepiandrosterone into androstenedione, and 3β, 17β-dihydroxy-Δ5-androstene into testosterone. The 3β-HSD

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Abbreviations used: BSA, bovine serum albumin; CORT, corticosterone; E_2, oestradiol; GC/MS, gas chromatography–mass spectrometry; HFBA, heptafluorobutyric anhydride; 3β-HSD, 3β-hydroxysteroid dehydrogenase; IC_{50}, 50% inhibition concentration; PREG, pregnenolone; PROG, progesterone; TEST, testosterone; 3β,5β-THA, 3β-hydroxy-5β-androstane-17-one.

isoenzymes are expressed not only in classical steroidogenic tissues, namely the adrenal cortex, testis, ovary and placenta, but also in other tissues including liver, skin, kidney and the nervous system. Four different isoforms of rat 3β-HSD cDNAs, which share more than 80% nucleotide identity, have been characterized so far (Lorence *et al*. 1991; Naville *et al*. 1991; Simard *et al*. 1991; Zhao *et al*. 1991; Labrie *et al*. 1992; Mason 1993; Simard *et al*. 1993).

The most important control of steroid hormone biosynthesis in the adrenal gland, ovary and testis is related to peptidic hormones of pituitary origin. Nonetheless, local control mechanisms are also operative within these tissues. They involve the actions of the steroid hormones themselves. Thus, inhibition of steroidogenic enzymes by the steroid product or by other steroid hormones may be an important mechanism in the control of steroidogenesis. For example, oestradiol acts as a competitive inhibitor of the 3β-HSD enzyme in MA-10 Leydig tumour cells (Freeman 1985). The involvement of steroid hormones in the regulation of PROG biosynthesis has also been documented in placenta (Gower and Cooke 1983; Raimondi *et al*. 1989). In contrast to the steroidogenic endocrine glands, little is known concerning the regulation of steroid synthesis in the nervous system, which involves interactions between different cells. For example, astrocytes convert ^{3}H-labelled PREG to ^{3}H-labelled PROG when they are cultured at a low density and to ^{3}H-labelled 7α-hydroxy-PREG at confluency (Akwa *et al*. 1993). Recently, we have shown that the expression and activity of 3β-HSD in Schwann cells are regulated by axonal signals (Robert *et al*. 2001). However, the nature of the factors involved in the regulation of PROG synthesis remain to be identified. Among others, growth factors, neurotransmitters, neuropeptides and steroids are good candidates.

The main aims of this paper were to demonstrate the presence and to describe the kinetic properties of 3β-HSD activity in the sciatic nerve of male rats and to explore the effects of several steroids on its activity.

Materials and methods

Drugs and chemicals

[7-^{3}H(N)]-Pregnenolone (25 Ci/mmol) was purchased from Amersham (Orsay, France). PREG, PROG, oestradiol (E_2) corticosterone (CORT), testosterone (TEST), 3β-hydroxy-5β-androstane-17-one; the β-cyclodextrin, NAD^+, bovine serum albumin (BSA) and protease inhibitors were purchased from Sigma-Aldrich (St Louis, MO, USA). The solvents were of analytical grade from Carlo Erba (Milan, Italy). The derivatization reagent heptafluorobutyric anhydride (HFBA) was purchased from Pierce (Rockford, IL, USA). All other reagents used were of analytical grade.

Experimental animals

Male Sprague-Dawley rats 200–250 g were housed under standard laboratory conditions with a 12-h light : dark cycle and with food and water *ad libitum*. Animals were killed by decapitation between 10.00 h and 15.00 h. All procedures concerning animal care and use were carried out according to the European Community Council Directive (86/609/EEC).

Homogenate preparation

Adrenal glands (200–230 mg) and sciatic nerves (60–70 mg) of individual animals were quickly dissected. Tissues were rinsed, kept in 200 μL of ice-cold homogenization buffer and were processed within 15 min. The homogenization buffer used was 100 mM potassium phosphate pH 7.4; 1.5 mM EDTA, 20% glycerol, 1 mM phenylmethylsulphonyl fluoride containing three other freshly added protease inhibitors: peptastin A, antipain and leupeptin at a final concentration of 5 μg/mL for each inhibitor. Sciatic nerves were minced into small fragments and homogenization was performed with a glass–glass motor-driven homogenizer. The homogenates (500 μL) were centrifuged for 30 min at 1000 *g* at 4°C. The obtained supernatant was divided into aliquots, quickly frozen in dry-ice-cooled ethanol and stored at −70°C until the determination of protein concentrations and enzymatic activity. Samples were assayed within 48 h after preparation. All steps prior to freezing were carried out at 4°C. Protein concentrations were measured according to Bradford (1976) using BSA as a standard.

Assay of 3β-HSD activity in sciatic nerve homogenates

The activity of the 3β-HSD was determined according to a previously described protocol with some modifications (Couet *et al*. 1992). The rate of enzymatic reaction was calculated by evaluating the rate of conversion of ^{3}H-labelled PREG to ^{3}H-labelled PROG. The optimal conditions for the assay as a function of protein concentration (0.1–5 mg/mL), reaction time (5–240 min), and NAD^+ concentration (0.01–4 mM) were first determined. Briefly, samples of 50 μL from supernatants of sciatic nerve homogenates were incubated at 37°C in a total volume of 75 μL, containing NAD^+ as a co-factor and 1 μM of ^{3}H-labelled PREG as a substrate. The enzymatic reaction was stopped by chilling the incubation mixture in an ice–water slurry and by adding 250 μL ethyl acetate–isooctane (50 : 50 v/v). The mixture was shaken by using a multisample vortex, spun (5 min/each), and frozen in a dry-ice–ethanol bath. The liquid organic phase was transferred to another vial and evaporated, whereas the remaining aqueous fraction was re-extracted twice at room temperature, with the same organic mixture. The evaporated organic extracts were finally resuspended in 25 μL ethyl acetate. The PROG produced was separated from PREG by TLC on silica gel plates (60-f254, Merck, Strasbourg, France) using chloroform/ethyl-acetate (80 : 20 v/v) as solvent system. The substrate and the formed steroids were identified by co-migration on each TLC plate with the appropriate labelled standard steroids. Radioactivity was determined using an automatic lecturer (Berthold-Wallac, Finland) or by cutting the corresponding steroid spots and by determining the radioactivity in a scintillation counter.

Steroid analysis by gas chromatography–mass spectrometry

One set of animals was used to identify specifically PROG as the product generated by the bioconversion of PREG. For this, gas chromatography associated with mass spectrometry (GC/MS) was used. GC/MS is a novel analytical method that allows the measurement of small amounts of steroids with high specificity

and sensitivity (Liere *et al*. 2000). For this method, supernatants of sciatic nerve homogenates were incubated as described above but with non-radioactive PREG. Briefly, samples obtained by extraction with ethyl acetate–isooctane (50 : 50) were evaporated to dryness and resuspended in 1 mL of methanol/water (40 : 60 v/v). Then, samples were purified by solid-phase extraction on IST C18 minicolumns and submitted to HPLC by using a Lichrosorb diol column. PREG, PROG, and 3β-hydroxy-5β-androstane-17-one (3β,5β-THA) used as internal standard were collected between 10 and 29 min. The steroids from the HPLC fractions were derivatized using HFBA as a reagent. Calibration solutions and derivatized samples were injected with an AS800 autosampler in a GC 8000 Top chromatograph (Carlo Erba, Milan, Italy) coupled to a mass spectrometer (Automass 150, Finnigan Automass, Argenteuil, France). Steroid identification was performed in the full-scan mode in the mass/charge (*m/z*) range of 50–550 Da. Quantification was carried out in the single-ion monitoring modes, that is, only the *m/z* 298 Da (PREG-HFB), 510 Da (PROG-HFB) and 486 Da (3β,5β-THA-HFB) diagnostic ions, were detected in the line scale of elution of each steroid.

Kinetic analysis and regulation of 3β-HSD activity

Kinetic analysis

The 3β-HSD activity was determined by evaluating the rate of conversion of ^{3}H-labelled PREG to ^{3}H-labelled PROG. Incubations were carried out for 10 min at 37°C using different concentrations of ^{3}H-labelled PREG (0.03–4 μM). Protein concentration was adjusted to 2 mg/mL so that the rate of PROG formation was linear. The quantity of product formed was calculated from the known specific activity of the substrate, the reaction velocity and the information obtained from the automatic lecturer. After the appropriate corrections for recovery, the values of K_m and V_{max} were estimated by Lineweaver–Burk plot analysis (Lineweaver and Burk 1934). Four independent assays using six different homogenates were carried out to determine the kinetic constants for PROG formation. All points were made in duplicate and at least six different concentrations of ^{3}H-labelled PREG were used in each assay.

Inhibition by trilostane

The effect of trilostane on the 3β-HSD activity was evaluated in three independent homogenates using increasing concentrations of trilostane (1–25 μM). Supernatants of sciatic nerve homogenates, were incubated with ^{3}H-labelled PREG (1 μM) and NAD^+ (1 mM), at 37°C for 1 h.

Regulation by steroids

The effect of increasing concentrations of steroids on the conversion rate of PREG to PROG was studied in three different experiments. Supernatants of sciatic nerve homogenates from two or three different animals were mixed and used for each experiment. Steroid effects were assessed by the addition to the homogenates of 10 μL concentrated steroid in ethanol. Control tubes (100% of conversion) contained 10 μL ethanol. The range of concentrations used were: E_2 (0.01–1 μM); TEST (0.01–50 μM); PROG (1–50 μM) and CORT (0.01–50 μM). The concentrations that produced 50% inhibition (IC_{50}) were obtained by the logit/log transformation.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). The comparative analysis of PREG and PROG levels by TLC or GC/MS was performed by statistical correlation calculated using a computer-assisted program (STATVIEW 4.5; Abacus Concepts Inc., Berkley, CA, USA). The IC_{50} was calculated by linear regression of the logit/log transformation from the respective displacement curves (Rodbard and Lewalds 1979). K_m and V_{max} values were calculated by linear regression using a computer assisted program.

Results

Established conditions published elsewhere (Couet *et al*. 1992) were initially used to assess the activity of the 3β-HSD in the homogenates prepared from sciatic nerves of intact male rats (two sciatic nerves from one animal per homogenate). Tubes containing the supernatant of adrenal gland homogenates (two adrenal glands per homogenate) were included as a positive control of enzymatic activity. After 1 h of incubation in the presence of 1 mM NAD^+ and 1 μM ^{3}H-labelled PREG, the supernatant of sciatic nerve homogenates (2 mg/mL protein) showed large individual differences in 3β-HSD activity, whereas enzyme activity in the adrenal gland supernatants did not significantly differ among animals. Indeed, the rate of ^{3}H-labelled PREG to ^{3}H-labelled PROG conversion was 80–90% for 10% of the samples, 20–50% for half of the samples and no activity could be detected in 40% of the homogenates. The observed variability was unlikely to be caused by the assay conditions as the different parameters such as tissue sampling, homogenization and incubation, were rigorously kept constant. Variability may thus reflect real differences in enzymatic activity between animals, which remains to be explained. In addition, the homogenates from the adrenal glands of the same animals showed high 3β-HSD activity and there was no correlation between the activity observed in the sciatic nerves and the adrenal glands. In this paper, we report the results corresponding to homogenates from sciatic nerves showing a conversion rate of PREG to PROG between 20 and 50%. The selection of samples according to this 'rate of conversion' was performed on the day of homogenate preparation by testing an aliquot of each supernatant.

The 3β-HSD activity, determined by the conversion of ^{3}H-labelled PREG to ^{3}H-labelled PROG, showed a direct relationship with the PREG concentrations in the incubation tubes. For PREG concentrations higher than 1 μM there was saturation of the enzyme (Fig. 1a). This activity also showed an increase as a function of protein concentration from 1 to 4 mg/mL (Fig. 1a). For the following experiments the amount of proteins was adjusted to a final concentration of 2 mg/mL and the amount of ^{3}H-labelled PREG used was 1 μM. For these concentrations the rate of PROG formed in 1 h was linear.

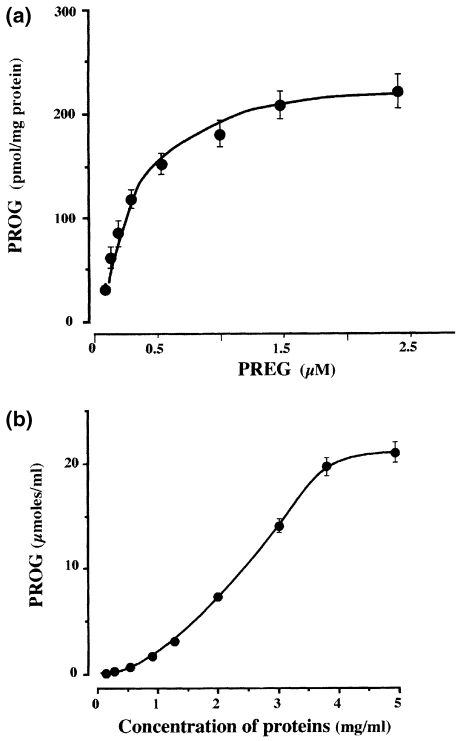

Fig. 1 (a) Conversion of PREG to PROG by supernatants of sciatic nerve homogenates using different concentrations of substrate (0.03–2.5 μM ^{3}H-labelled PREG). Incubations were performed at 37°C (2 mg protein/mL, 1 mM of NAD^+) for 10 min. Values were obtained from four independent assays and are expressed as mean ± SEM. (b) Changes in 3β-HSD activity as a function of protein concentration from 0.1 to 5 mg/mL. Incubations were performed at 37°C (1 μM ^{3}H-labelled PREG, 1 mM of NAD^+) for 1 h. Values were obtained from four different supernatants and are expressed as mean ± SEM. Omission of error bars indicates that the standard error is confined within the symbol.

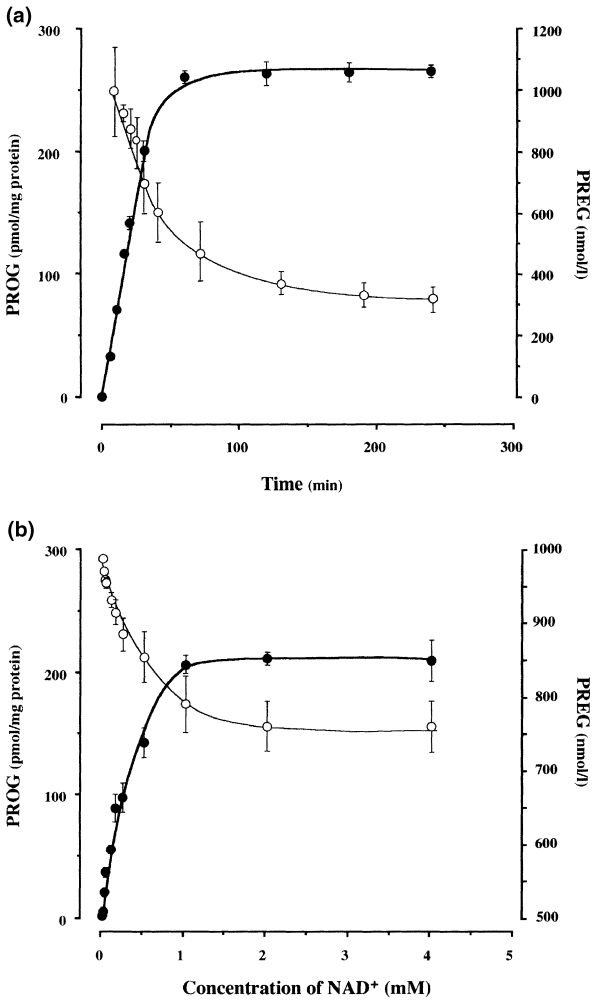

Fig. 2 Dependence of 3β-HSD activity on (a) incubation time and (b) the amount of cofactor (NAD^+) added to supernatants of rat sciatic nerve homogenates. Incubations were carried out at 37°C. For (a) the NAD^+ concentration used was 1 mM and for (b) the time of incubation was 1 h. Values represent the mean ± SEM of three to five independent assays. Omission of error bars indicates that the standard error is confined within the symbol. ●, PROG; ○, PREG.

Kinetic analysis

Different parameters involved in the enzymatic activity were evaluated. First, the time-course of PROG formation was assessed. As shown in Fig. 2(a), in a reaction mixture containing 2 mg/mL protein, 1 mM NAD^+ and 1 μM ^{3}H-labelled PREG, at 37°C, there was a rapid increase in the conversion of PREG to PROG between 5 and 50 min of incubation, whereas the amount of PROG formed remained constant after 1 h of incubation. Next, the effect of increasing concentrations of NAD^+ in the reaction mixture was studied. A proportional increase in the 3β-HSD activity was observed when the range of NAD^+ concentrations used was from 0.1 mM to 0.5 mM. The rate of PREG conversion to PROG was maximal at concentration of 1 mM NAD^+, as shown in Fig. 2(b).

Table 1 shows the results obtained by measuring the transformation of PREG to PROG in the supernatant of sciatic nerve homogenates from five intact animals. The levels of both steroids at the end of the incubation time, were usually calculated by extrapolation of the relationship of tritiated picks obtained by TLC to the initial amount of steroid added before incubation. On this table we also show the results obtained by GC/MS determination. The mass spectra of the derivatized steroids and the elution pattern are shown in Fig. 3. PROG reacting with the derivatization agent gave two different isomers. Only the 3,5-diene isomer, which is formed in major proportion (95%) has been used for quantification. This latter method allowed us to identify specifically PROG as the product generated by the bioconversion of PREG. The steroid level values obtained by the two methods, showed a significant correlation for PREG ($r^2 = 0.914$; $p < 0.02$) and PROG ($r^2 = 0.965$; $p < 0.003$).

Another set of experiments was carried out to determine the affinity of this enzyme for PREG by assessing the initial velocities at different concentrations of substrate, after 10 min of

Table 1 Levels of PREG and PROG present in the supernatants of five different homogenates of rat sciatic nerves determined by comparing two different methods, TLC and GC/MS

	PREG (pmol/sample)		PROG (pmol/sample)	
	TLC	GC/MS	TLC	GC/MS
Sample 1	49.1	47.1	22.7	24.5
Sample 2	62.9	58.7	9.0	13.2
Sample 3	61.2	62.2	10.3	12.0
Sample 4	64.0	62.4	7.6	9.3
Sample 5	65.5	62.7	5.8	9.1

Incubations (2 mg protein/mL, 1 mM NAD^+) were performed at 37°C for 1 h, using ^{3}H-labelled PREG as a substrate for analysis by TLC and non-radioactive PREG for analysis by GC/MS. Incubations and extraction of steroids were carried out in parallel for both methods. A high correlation between values obtained by both methods was found for the amounts of PREG ($r^2 = 0.914$) and PROG ($r^2 = 0.965$).

incubation. Results are shown in Fig. 4. The analysis of kinetic parameters by double-reciprocal plots of velocity against substrate concentration, resulted in a K_m value of 1.06 ± 0.19 μM and a V_{max} of 32.27 ± 4.05 nmol/min/mg protein.

Effect of trilostane on 3β-HSD activity

The effect of trilostane, a competitive inhibitor of steroid synthesis (Young *et al*. 1994; Cooke 1996), which particularly inhibits the enzymatic activity of the 3β-HSD (Potts *et al*. 1978), was also evaluated. Figure 5 shows the effect of this potent inhibitor on the rate of conversion of PREG to PROG in homogenates from sciatic nerves. Reduction of PROG synthesis by trilostane was dose dependent. The IC_{50} calculated after logit/log linearization was 4.06 ± 2.58 μM.

Inhibition of the 3β-HSD activity by steroids

Subsequent experiments have been performed to investigate the effects of different steroid hormones on the *in vitro*

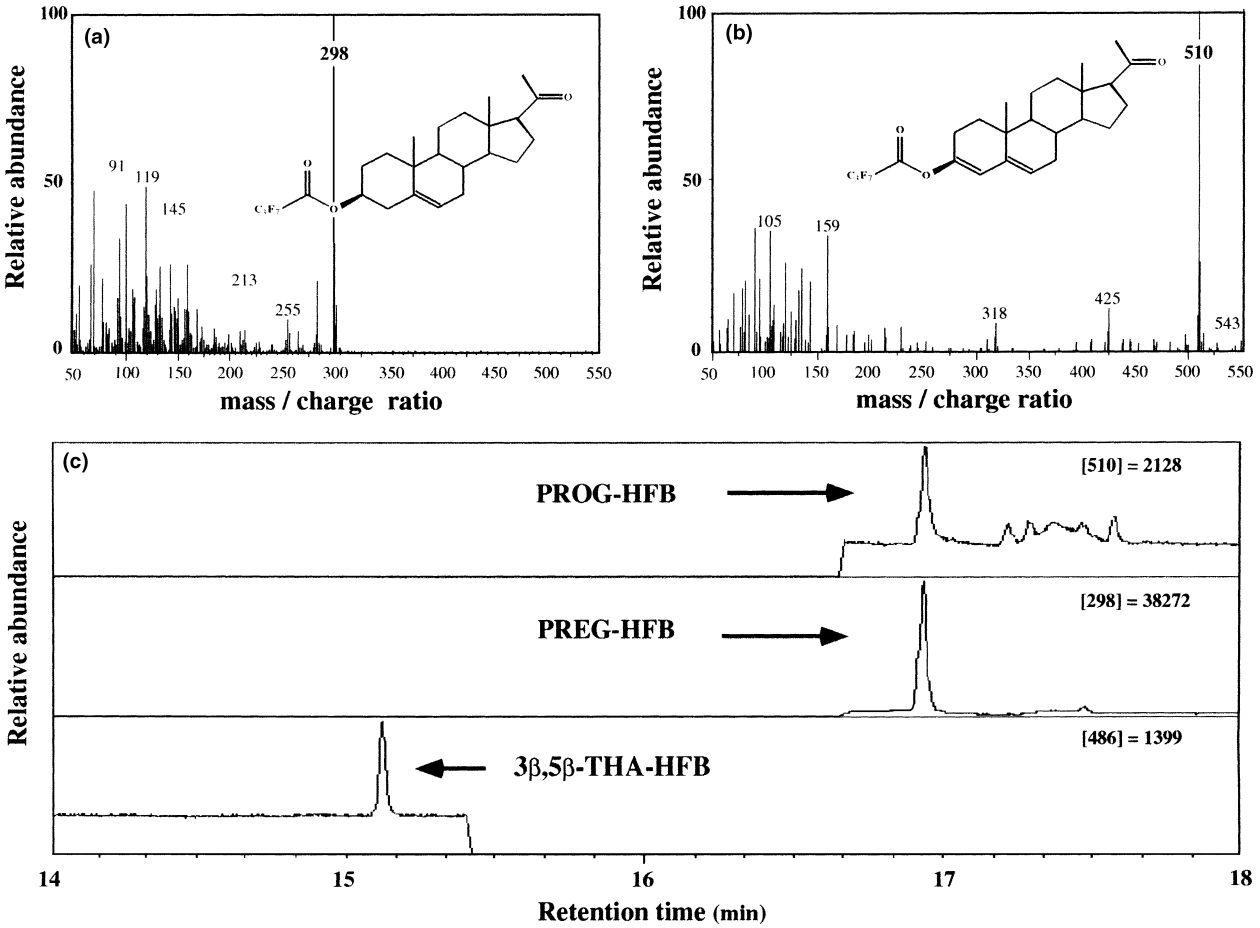

Fig. 3 Analysis of steroids by GC/MS: electron impact mass spectra of the derivatized steroids PREG-HFB (a) and PROG-HFB (b) and reconstructed ion chromatograms (c) of the steroids, PREG-HFB (m/z 298) and PROG-HFB (m/z 510) and the internal standard, 3β,5β-THA-HFB (m/z 486). Gas chromatography was performed in the splitless mode with a 1-min splitless-time. The mass spectrometer was operated in the electron impact mode with an emission current of 2000 μA and with an ionization energy of 70 eV.

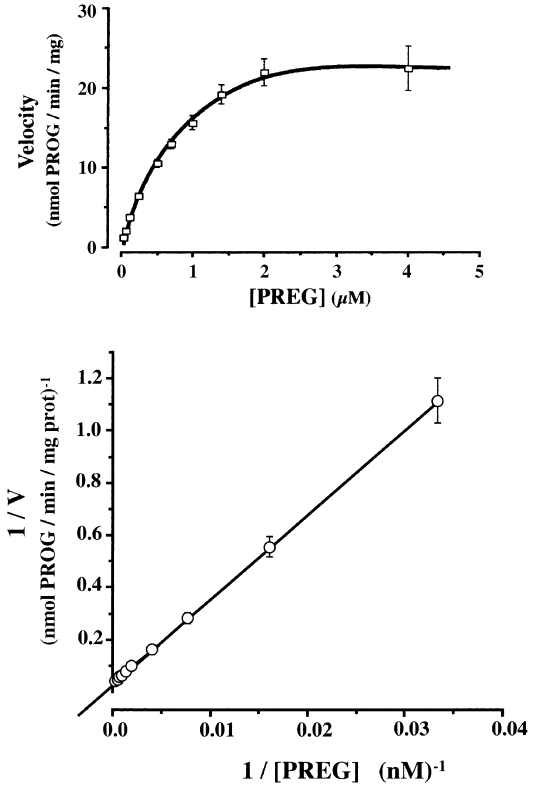

Fig. 4 Representative plot of the apparent Michaelis constant (K_m) and V_{max} calculated by the Lineweaver–Burk double reciprocal method. Four different experiments were performed with supernatants of sciatic nerve homogenate with a similar conversion rate of PREG to PROG. The reactions were initiated by addition of different concentrations of ^{3}H-labelled PREG (0.03–4 μM). Incubations, containing 1 mM of NAD^+ as a co-factor, were performed at 37°C for 10 min. In all the experiments, substrates and products were separated by TLC. The values obtained, expressed as mean ± SEM were: $K_m = 1.06 \pm 0.19$ μM; $V_{max} = 32.27 \pm 4.05$ nmol/min/mg protein.

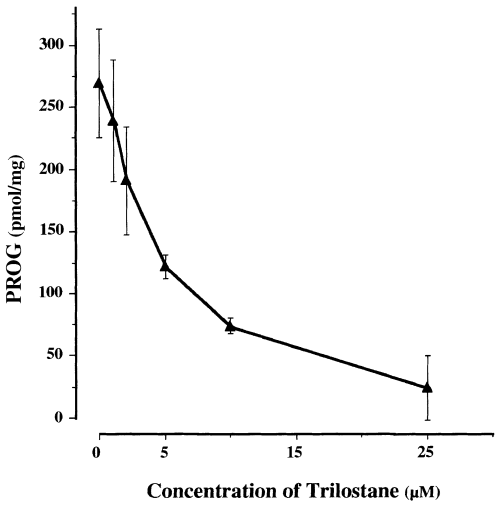

Fig. 5 Effect of increasing concentrations of trilostane on 3β-HSD activity in sciatic nerve. Supernatants of sciatic nerve homogenates (2 mg protein/mL) were incubated with 1 μM ^{3}H-labelled PREG and 1 mM NAD^+, at 37°C for 1 h, in the absence or presence of increasing concentrations of trilostane (1–25 μM). Steroids were separated by TLC. The IC_{50} obtained by logit/log was: 4.06 ± 2.58 μM. Data were expressed as the mean ± SEM from duplicates of three independent assays.

conversion of PREG to PROG by sciatic nerve homogenates. The activity of 3β-HSD was determined in the presence or absence of E_2, TEST, CORT, or PROG. The steroids tested are either end-products of the enzyme or may control the PROG formation by short feedback regulation (Gower and Cooke 1983). Results are shown in Fig. 6. E_2 and TESTO were more efficient in inhibiting 3β-HSD activity than PROG and CORT. The steroid concentrations that produced IC_{50} of PROG biosynthesis were: 0.24 ± 0.03 μM for E_2; 8.58 ± 2.56 μM for TEST; 25.9 ± 1.3 μM for PROG and 80.0 ± 13.3 μM for CORT.

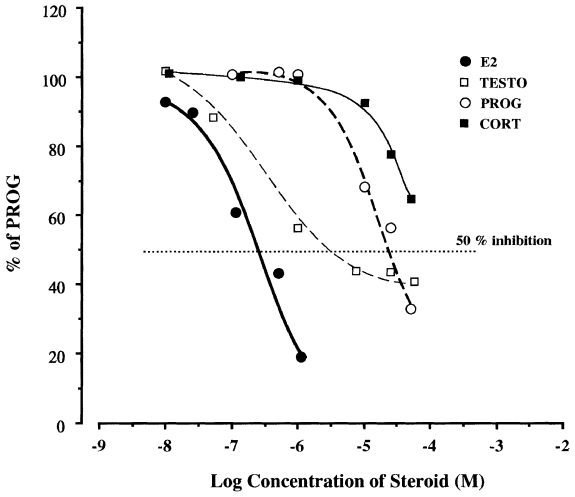

Fig. 6 Effect of increasing concentrations of different steroids on the conversion of PREG to PROG by the supernatants of rat sciatic nerves homogenates. Oestradiol (E_2; 0.01–1 μM), testosterone (TEST; 0.01–50 μM), progesterone (PROG; 1–50 μM) and corticosterone (CORT; 0.01–50 μM). Supernatants (2 mg protein/mL) were incubated with 1 μM ^{3}H-labelled PREG and 1 mM NAD^+ at 37°C for 1 h. The values of IC_{50} obtained by logit/log were $E_2 = 0.24 \pm 0.03$ μM; TEST = 8.58 ± 2.56 μM; PROG = 25.9 ± 1.3 μM; CORT = 80.0 ± 13.3 μM. Data are expressed as the mean of three different supernatants for E_2 and PROG and of two supernatants for TEST and CORT.

Discussion

The present paper demonstrates for the first time the activity of 3β-HSD in the sciatic nerve. It assesses the kinetic parameters of 3β-HSD activity in the rat sciatic nerve and shows the regulatory effects of some steroids. The expression

of 3β-HSD mRNA and protein have been previously shown in the sciatic nerve as well as in Schwann cells (Guennoun *et al*. 1997; Robert *et al*. 2001), but nothing was known about the enzymatic activity and its kinetic parameters in peripheral nervous tissue. In sciatic nerve, the apparent K_m for PREG conversion to PROG was 1.06 ± 0.19 μM. This value is similar to that previously reported for the rat type-I and type-IV isoforms of the 3β-HSD and it is 10 times lower than the K_m of the type-II isoform (Simard *et al*. 1991, 1993). Our results thus suggest that rat 3β-HSD type-II isoform, that is the principal 3β-HSD isoform expressed in adrenal cortex and gonads, is not the active isoform in rat sciatic nerve. Indeed, the type-II isoform shows a reduced affinity for PREG with K_m value of 14 μM (Simard *et al*. 1991). The high K_m value of type-II 3β-HSD isoform, mainly expressed in steroidogenic tissues could be related to the high levels of endogenous substrates present in these classical steroidogenic glands. Meanwhile the low K_m value estimated in the sciatic nerve, similar to the K_m for type-I and type-IV isoforms, which are preferentially expressed in peripheral tissues, could greatly facilitate PROG formation from the relatively low concentration of PREG usually present in the sciatic nerve.

In sciatic nerve homogenates, both E_2 and TEST act as inhibitors of the 3β-HSD activity. There is an increasing body of evidence supporting the idea that steroids may regulate their biosynthesis. E_2 inhibits PROG production by decreasing the 3β-HSD activity in porcine theca cells (Tonetta *et al*. 1987), human fetal adrenal cells (Fujieda *et al*. 1982), and MA-10 Leydig tumour cells (Freeman 1985). It has also been reported that PROG decreases the 3β-HSD activity in porcine theca cells (Tonetta *et al*. 1987). E_2, TEST and PROG have been shown to decrease the 3β-HSD activity in tissue fragments and microsomes from human term placenta (Raimondi *et al*. 1989). Therefore, these studies as well as our results suggest that steroids may regulate PROG biosynthesis directly by controlling the activity of the 3β-HSD enzyme in different tissues, including the peripheral nerves. Since the inhibition of 3β-HSD activity by E_2, TEST and PROG occurs in the supernatants of sciatic nerve homogenates, that is in a cell-free system, it suggests a direct inhibitory effect that is not mediated by a steroid receptor. However, high concentrations of steroid were required to inhibit 3β-HSD activity and the physiological significance of these *in vitro* observations remains to be established.

The regulation of PROG synthesis in the peripheral nervous system remains largely unexplored, except for a recent study showing a role for cellular interactions between Schwann cells and sensory neurons. Indeed, we have shown that mRNA expression and activity of 3β-HSD are induced in Schwann cells by sensory neurons (Robert *et al*. 2001). The neuronal factors involved in this regulation remain to be identified. The present study shows that some steroids, in particular E_2 and TEST, may regulate PROG biosynthesis in sciatic nerves as has been demonstrated for the endocrine glands and placenta.

In vivo, the activity of 3β-HSD would be a function of the concentration and of the K_i of many competing steroids, of the substrate concentration, as well as of the K_m and V_{max} of the enzyme. Changes in steroid concentrations could affect the 3β-HSD activity, particularly in the absence of adaptive changes in substrate concentration. At the normal physiological conditions in male rats, circulating plasma concentrations of PROG, E_2 and TEST are not high enough to exert a pronounced inhibitory effect on 3β-HSD activity similar to that shown here. However, in some tissues, the internal concentrations of steroids may be much higher than in circulating plasma. In addition, in some conditions, such as during the oestrous cycle, during pregnancy, or under conditions of stress, the local tissue concentrations of steroids may reach high levels sufficient to inhibit 3β-HSD activity.

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