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Olea europaea leaf extract exerts L-type Ca^{2+} channel antagonistic effects

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ABSTRACT

Ethnopharmacological relevance: In Southern Europe *Olea europaea* leafs are known as a folk remedy for hypertension. Cardiovascular diseases are still the leading causes of morbidity and mortality in industrialized countries with hypertension being one of the main risk factors.
Aim of the study: We investigated effects of a commercial *Olea europaea* leaf extract (OLE) on isolated hearts and cultured cardiomyocytes.
Materials and methods: Isolated rabbit hearts were perfused according to the Langendorff technique and connected to a 256-channel epicardial mapping system. Voltage clamp experiments were performed in cultured neonatal rat cardiomyocytes using a perforated-patch technique.
Results: OLE caused a concentration-depended decrease in systolic left ventricular pressure and heart rate as well as an increase in relative coronary flow and a slight, but not significant prolongation of PQ-time. There were no significant changes between the groups in the activation-recovery interval and its dispersion, total activation time, peak-to-peak amplitude, percentage of identical breakthrough-points and similar vectors of local activation. Voltage clamp experiments in cultured neonatal rat cardiomyocytes showed a significant decrease in maximum $I_{Ca,L}$ by OLE which was reversible upon wash-out.
Conclusions: OLE suppresses the L-type calcium channel directly and reversibly. Our findings might help to understand the traditional use of OLE in the treatment of cardiovascular disease.

1. Introduction

Cardiovascular diseases are still the leading causes of morbidity and mortality in industrialized countries and high blood pressure is one of the main risk factors (WHO, 2002). Though it is not a problem of modern life, because cardiovascular diseases had been known also hundreds of years ago, just as long as mankind had searched for treatments against them. In history plants had been the most important source for remedies and plant-derived drugs gained in importance in the treatment of cardiovascular diseases, e.g., digitalis and digitoxin derived from the foxglove or amiodarone derived from amni visnaga. In recent years there was not only a rise in public interest in naturoceuticals, but also clinical and pharmaceutical researchers rediscovered traditionally used phytotherapy as a source for new therapeutical approaches (Hermann, 2002; Gilani and Rahman, 2005).

The olive tree (*Olea europaea*) has been cultivated in the Mediterranean for more than a thousand years. The positive effects on health of its fruits and oil are well known and several studies have shown a relation between Mediterranean diet and lower risks of cardiovascular diseases (Keys et al., 1986; Trichopoulou et al., 1995). Olive oil as the main fatty component is considered as one of the most important health promoting factors in Mediterranean diet. It mediates positive effects on cardiovascular and metabolic diseases, inflammatory and autoimmune diseases as well as in prevention of breast and colon cancer (Alarcón de la Lastra et al., 2001).

Not only the olive oil, but also the leaves have been used for medical purposes, and were introduced recently into the Pharmacopoea PhEur 5. Especially in Southern Europe they are known as a folk remedy for hypertension and diabetes (Komaki et al., 2003).

Olive leaves contain different groups of constituents, such as iridoids, polyphenols, flavones and carbohydrates (Gariboldi et al., 1986; Heimler et al., 1992; Le Tutour and Guedon, 1992; Romani et al., 1994; Pieroni et al., 1996). Oleuropein, a phenolic secoiridoide, is used as the typical marker compound of extracts (as recently in PhEur 5) and its concentration is significantly higher in leafs than the fruits or oil. The vasodilatative (Zarzuelo et al., 1991), hypotensive (Lasserre et al., 1983) and hypoglycemic (Gonzalez et al., 1992) effects of olive leafs have been shown. Gilani et al. have found indications that the hypotensive effect of a crude extract from olive fruit may be mediated via calcium channel antagonism by using

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indirect methods (Gilani et al., 2005), but there are still open questions concerning the molecular mechanism of action. Since the molecular mechanism and the type of cardiovascular action of olive *leaf* extracts are still unknown, we investigated the effects of *Olea europaea* leaf extract on mammalian hearts and cellular electrophysiology.

2. Materials and methods

All animal experiments were performed in accordance with the German law on animal welfare and were approved by the local committee for animal welfare.

2.1. Heart preparation and epicardial mapping

The method has been described in more detail previously (Dhein et al., 1990, 1993) and will be explained only briefly in the following paragraph. Male white New Zealand rabbits (conventional, normally fed ad libitum, 1800–2000 g) were anesthetized with ketamine and xylazine i.m. and treated with 1000 IU/kg heparin i.v. 5 min before they were killed by exsanguination. The heart was excised, prepared and perfused according to the Langendorff technique with Tyrode's solution (constant pressure 70 cm H_2O). For epicardial mapping the hearts were connected to a 256-channel mapping system HAL 3 (ELSA, Aachen, Germany, temporal resolution: 4 kHz per channel, amplitude resolution: 0.04 mV, interchannel coupling < −60 dB, bandwidth of the system: 0.5–20 kHz, data were not filtered). Two hundred and fifty-six AgCl electrodes were cast in 4 polyester plates (in 8 × 8 orthogonal matrices with 1 mm interelectrodes distance) which were attached to the hearts surface in an elastic manner, so that they could follow the hearts movements easily without dislocation (Dhein et al., 1993). The hearts were spontaneously beating during the entire experiments. After 30 min of equilibration *Olea europaea* leaf extract was applied in a cumulative manner (final concentrations of oleuropein 1, 10, 20 and 50 μM) and each concentration was applied for 15 min before data acquisition. We performed $n = 5$ samples as well as $n = 4$ control experiments without any drug application.

The common functional parameters left ventricular pressure (LVP), coronary flow (CF) and heart rate (HR) were assessed continuously. Left ventricular pressure was measured by an intraventricular balloon and coronary flow was determined volumetrically by collecting the coronary effluent (Dhein et al., 1993).

For mapping data evaluation the activation and repolarization time points were determined as *t*(dU/dtmin) or *t*(dU/dtmax) at each electrode (Durrer and van der Tweel, 1954; Millar et al., 1985; Spach and Dolber, 1986; Dhein et al., 1993). Time points were calculated automatically and thereafter controlled manually by the experimenter. From these data activation-recovery interval (ARI), reflecting the local epicardial action potential duration, was calculated for each electrode. The distribution of the activation recovery intervals was analyzed for each area of the heart (front, back, left and right wall) by calculating the standard deviation of ARI (ARI-dispersion, DISP) at 64 electrodes.

We determined those electrodes which were activated before any of the neighbouring ones and defined them as breakthrough-points, which can be considered as the origins of epicardial activation (Arisi et al., 1983). Breakthrough-points for heart beats under treatment were compared to those under control conditions by calculating the percentage of breakthrough-points with identical location (BTP, deviating not more than 1 mm from their location under control conditions). However identical heartbeats occur only rarely. From previous studies it is known, that proarrhythmic stimuli reduce BTP similarity and that the critical value for the occurrence of arrhythmia is a reduction below 50% BTP similarity (Dhein et al., 1988, 1990, 1993).

Analysis of epicardial excitation was performed by calculating an activation vector for each electrode from the activation times and the locations of the surrounding electrodes which were activated after the central electrode (Müller et al., 1991). This allows us a quantitative and comparative description of the activation process by vectors which give direction and velocity of local activation. The percentage of similar vectors (VEC, deviating not more than 5°) between heart beats under treatment compared with those under control conditions is determined. A VEC similarity below 10% is considered as the critical value according to induction of arrhythmia (Dhein et al., 1988, 1993).

Moreover, total activation time (TAT) for each area was calculated as the delay between the activation of the first and activation of the last electrode. As an indirect indicator of the velocity of the activation wave in the tissue beneath the electrode (Spach and Dolber, 1986), the amplitude of the ventricular activation complex was assessed as the peak-to-peak amplitude (PTP).

2.2. Cell culture

Cardiomyocytes were isolated and cultured according to the following protocol: Neonatal Spraque–Dawley rats of either sex at age of maximum 24 h were sacrificed by decapitation. The ventricles were digested in collagenase type II solution and after centrifugation the pellet was resuspended in first day medium. To reduce the number of fibroblast there was a pre-plating period. The cells were seeded in petri dishes on cover slips coated with 1% gelatin and incubated at 37 °C and 5% CO_2. Medium was changed 24 h after seeding and then every other day.

2.3. Whole cell patch clamp experiments

Patch clamp experiments were performed on days 1–4 after cell preparation. Therefore the cover slips on which the cells are adherent were transferred to an experiment chamber (1 ml) mounted on an inverted microscope (Axiovert, Zeiss, Jena, Germany). The cells were continuously superfused with extracellular solution which is heated shortly before inflow so that all experiments could be performed at 36 °C. L-type calcium channel currents were recorded using the amphotericin B perforated patch technique to reduce rundown of $I_{Ca,L}$ (Hoppe et al., 2005). We used a discontinuous patch clamp amplifier (SEC 05, npi electronic, Tamm, Germany), which allows accurate measurements independent from serial resistance, and CellWorks software (npi electronic). For analysis data were low-pass filtered at 1 kHz. Electrodes were pulled from borsilicate glass capillaries with inner filament and the resistances were 4–6 MΩ. For perforated patch, we dissolved 3 mg of amphotericin B in 100 μl of dimethyl sulfoxide as a stock solution which can be stored at −20 °C for about a week. Four microliters of this solution were added to 0.5 ml of the electrode solution shortly before the experiments, resulting in a final concentration of 240 μmol amphotericin B (Rae et al., 1991) in the electrode solution. The electrode tips were immersed in normal electrode solution for 1 s and then backfilled with the amphotericin B containing solution. When a cell-attached configuration was achieved, the cells were kept at a holding potential of −40 mV and then we waited 10–15 min until we could elicit a clear Na^+-channel current as a quality control of break-in and cell control. Thereafter the potential of the cell was changed stepwise from −40 to +100 mV for 200 ms in 10 mV steps. Data was recorded every 2 min until 90 min. For analysis $I_{Ca,L}$ from the beginning of the experiment was compared to $I_{Ca,L}$ 20 min after wash-in of *Olea europaea* leaf extract at a final concentration of 20 μmol

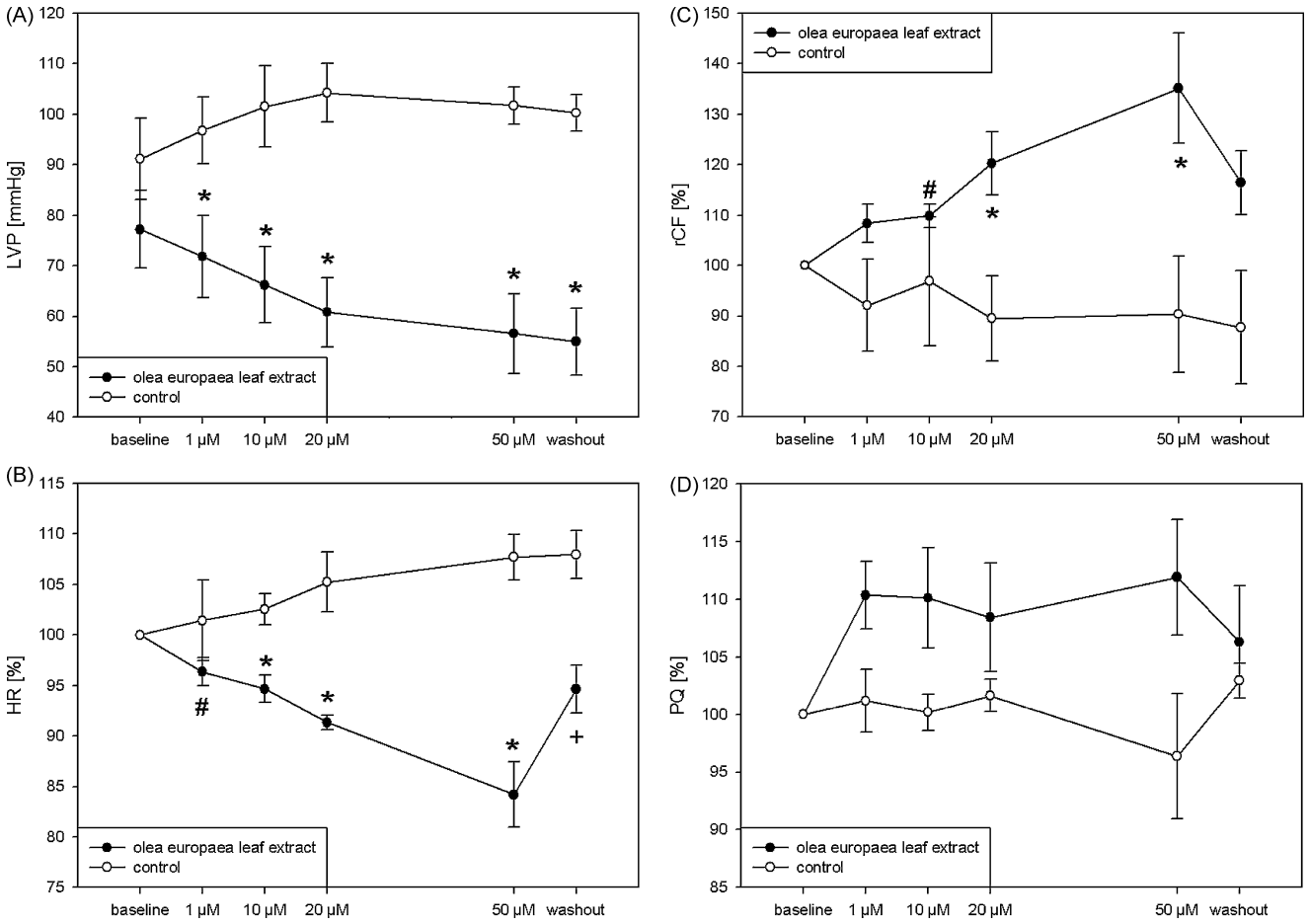

Fig. 1. (A) Left ventricular pressure (LVP) in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 5$). There was a significant reduction in LVP at all the tested concentrations of oleuropein. *Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data as well as a significant difference *Olea europaea* leaf extract vs. baseline of olea group. (B) Heart rate (HR) in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 5$) given as percentages of baseline phase. There was a significant reduction in HR in addition of *Olea europaea* leaf extract containing oleuropein in final concentrations of 10, 20, and 50 μM. *Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data as well as a significant difference *Olea europaea* leaf extract vs. baseline of olea group. #Indicates a significant difference *Olea europaea* leaf extract vs. baseline of olea group. +Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data. (C) Ratio between CF and pressure rate product (LVP × HR) given as relative coronary flow (rCF) as percentage of baseline phase in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 5$). There was a significant increase in rCF in addition of *Olea europaea* leaf extract containing oleuropein in final concentrations of 10, 20, and 50 μM. *Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data as well as a significant difference *Olea europaea* leaf extract vs. baseline of olea group. #Indicates a significant difference *Olea europaea* leaf extract vs. baseline of olea group. (D) PQ-time (PQ) as percentage of baseline phase in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 3$). There were no significant changes in PQ in addition of *Olea europaea* leaf extract.

oleuropein and time-matched data of control experiments, respectively.

2.4. Composition of solutions [in mM]

2.4.1. Mapping

Tyrode's solution: NaCl 136.8, KCl 5.36, NaH_2PO_4 0.42, $NaHCO_3$ 23.8, $MgCl_2$ 1.05, $CaCl_2$ 1.8, glucose 11, pH 7.4 (equilibrated with 95% O_2 and 5% CO_2).

2.4.2. Cell culture

PBS-glucose: NaCl 137, KCl 2.68, Na_2HPO_4 8.3, KH_2PO_4 1.47, glucose 20. Isolation solution: NaCl 137, KCl 2.68, Na_2HPO_4 8.3, KH_2PO_4 1.47, glucose 20, albumin 10%, collagenase type II 200 U/ml. Stop medium: M199 (with Earle's Salts, GlutaMAX™ I), fetal calf serum 5%, penicillin 200 U/ml, streptomycin 200 μg/ml, HEPES 21, pH 7.4. First day medium: M199 (with Earle's salts, GlutaMAX™ I), fetal calf serum 5%, horse serum 10%, penicillin 200 U/ml, streptomycin 200 μg/ml, HEPES 21, pH 7.4. Medium for the following days: M199 (with Earle's salts, GlutaMAX™ I), fetal calf serum 1%, horse serum 10%, penicillin 200 U/ml, streptomycin 200 μg/ml, HEPES 21, pH 7.4.

2.4.3. Patch clamp

Extracellular solution: NaCl 135, $CsCl_2$ 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 10, pH 7.3; for wash-in 8 mg/100 ml *Olea europaea* leaf extract was added, this means a final concentration of 20 μmol/l oleuropein. Intracellular solution: Cs-Aspartat 120, $CsCl_2$ 20, $MgCl_2$ 1, NaCl, HEPES 10, EGTA 10, MgATP 2, NaGTP 0.1, pH 7.2; for backfilling 240 μmol/l amphotericin B was added.

2.5. Chemicals and plant extract

An 80% ethanolic *Olea europaea* leaf dry extract containing 13.4% oleuropein was obtained from Johannes Bürger Ysatfabrik (Bad Harzburg, Germany) certified by extract chemie (Stadthagen, Germany), charge no. 9687/01. The dry extract was dissolved in 1% DMSO before experiments. For further extract characterization see Section 3.

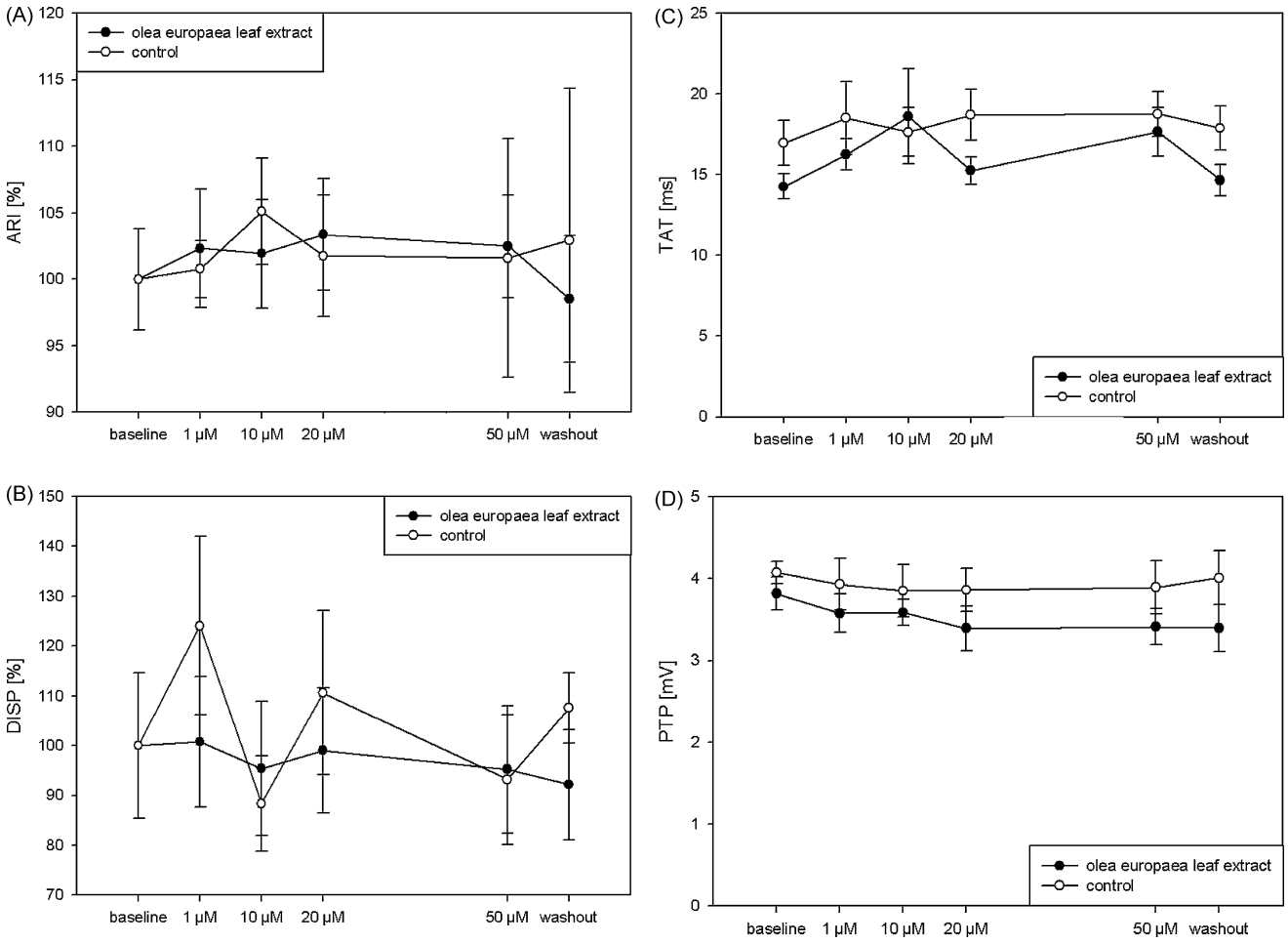

Fig. 2. (A) Activation-recovery interval (ARI) as percentage of baseline phase. (B) Dispersion of ARI (DISP) as percentage of baseline phase. (C) Total activation time (TAT). (D) Peak-to-peak amplitude (PTP). There were no significant changes in ARI, DISP, TAT or PTP in control group ($n = 4$) as well as in addition of *Olea europaea* leaf extract ($n = 5$).

All other chemicals were of the purest available quality and were purchased from Sigma–Aldrich (Munich, Germany) except collagenase type II, fetal calf serum (Biochrom, Berlin, Germany), horse serum and M199 with Earle's Salts, GlutaMAX™ I (Gibco®). Heparin was purchased from ratiopharm (Ulm, Germany).

2.6. HPLC characterization of the extract

We used a C-18 nucleosil 100-5, EC250/4 column with a C-18 nucleosil 100-5, CC8/4 precolumn for analysis together with a Waters 600 controller and pump, and a Waters UV-DAD and Waters tunable 486 absorbance detector. Measurements were performed at 280 nm using two mobile phases A and B (A: 15% acetonitrile, 85% of a 2.5% acetic acid watery solution; B: 80% acetonitrile, 20% of a 2.5% acetic acid watery solution). Extract was analyzed using a gradient elution (time [minutes]/mobile phase A [% V/V]/mobile phase B [%V/V]: 0–5/85–80/15–20; 5–13/80–78/20–22; 13–15/78–60/22–40; 15–18/60–60/40–40; 18–20/60–50/40–50; 20–25/50–85/50–15; flow rate: 1 ml/min, temperature: 38 °C; injection volume: 20 µl).

2.7. Statistical analysis

All data are presented as mean ± standard error of mean. Statistical evaluation was performed by using ANOVA and if ANOVA indicated significant differences by *t*-test for paired or unpaired observations. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Epicardial mapping experiments

Our experiments have shown that *Olea europaea* leaf extract leads to vasodilatation, bradycardia and negative inotropy and possibly to delay of atrioventricular conduction.

The extract caused a concentration-dependent decrease in systolic left ventricular pressure (LVP) of 7.7% (1 µM) to 27.4% (50 µM). In a time-matched control series there was no reduction in LVP during the correspondent time interval. In parallel with that the relative coronary flow, given as ratio between CF and PRP (pressure rate product = LVP × HR), was increased, while in time-matched controls there was a slight but not significant decrease. Furthermore, there was a slowing in heart rate (HR) of 5.4% (10 µM) to 15.8% (50 µM) under *Olea europaea* leaf extract, while this parameter in time-matched controls exhibited a slight but not significant increase (Fig. 1A–C).

There was a delay of atrioventricular conduction given as prolongation of PQ-time, but the observed effect (maximum increase of PQ of 12% [50 µM]) was not significant for only $n = 3$ samples could be included in the analysis (Fig. 1D). There were no changes in PQ in time-matched controls.

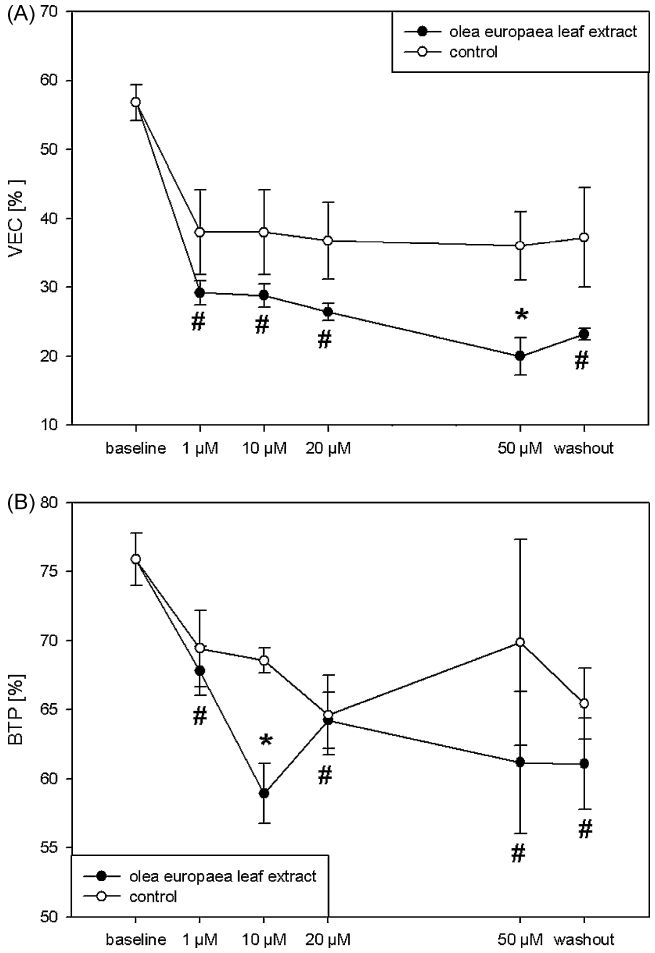

Fig. 3. (A) Percentages of similar VECs in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 5$). There was a significant reduction in VEC similarity in addition of *Olea europaea* leaf extract with a significant difference to time-matched control data at a concentration of 50 μM oleuropein. *Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data as well as a significant difference *Olea europaea* leaf extract vs. baseline of olea group. #Indicates a significant difference *Olea europaea* leaf extract vs. baseline of olea group. (B) Percentages of identical breakthrough-points (BTP) in controls ($n = 4$) and in addition of *Olea europaea* leaf extract ($n = 5$). There was a significant reduction in BTP similarity in addition of *Olea europaea* leaf extract with a significant difference to time-matched control data at a concentration of 20 μM oleuropein. *Indicates a significant difference *Olea europaea* leaf extract vs. time-matched control data as well as a significant difference *Olea europaea* leaf extract vs. baseline of olea group. #Indicates a significant difference *Olea europaea* leaf extract vs. baseline of olea group.

Neither in controls nor in addition of *Olea europaea* leaf extract significant changes in ARI, dispersion of ARI (DISP), TAT, PTP were observed (Fig. 2A–D).

The percentage of identical BTPs and similar VECs compared with heartbeats under control conditions was diminished significantly at all concentrations. In comparison to time-matched control data VEC and BTP values were slightly reduced but reaching significance only at 10 μM (BTP) or 50 μM (VEC).

However, these reductions of BTP and VEC similarity were not below the critical values of 50% (minimum 58.95 ± 2.19% [10 μM]) and 10% (minimum 20.0 ± 2.74% [50 μM]), respectively (Fig. 3).

The results of our mapping experiments, especially the observed negative inotropy, bradycardia and vasodilatation in absence of effects on ARI or TAT, lead us to the assumption of *Olea europaea* leaf extract possibly acting as an antagonist on L-type calcium channel. Therefore, we performed voltage clamp experiments and tested

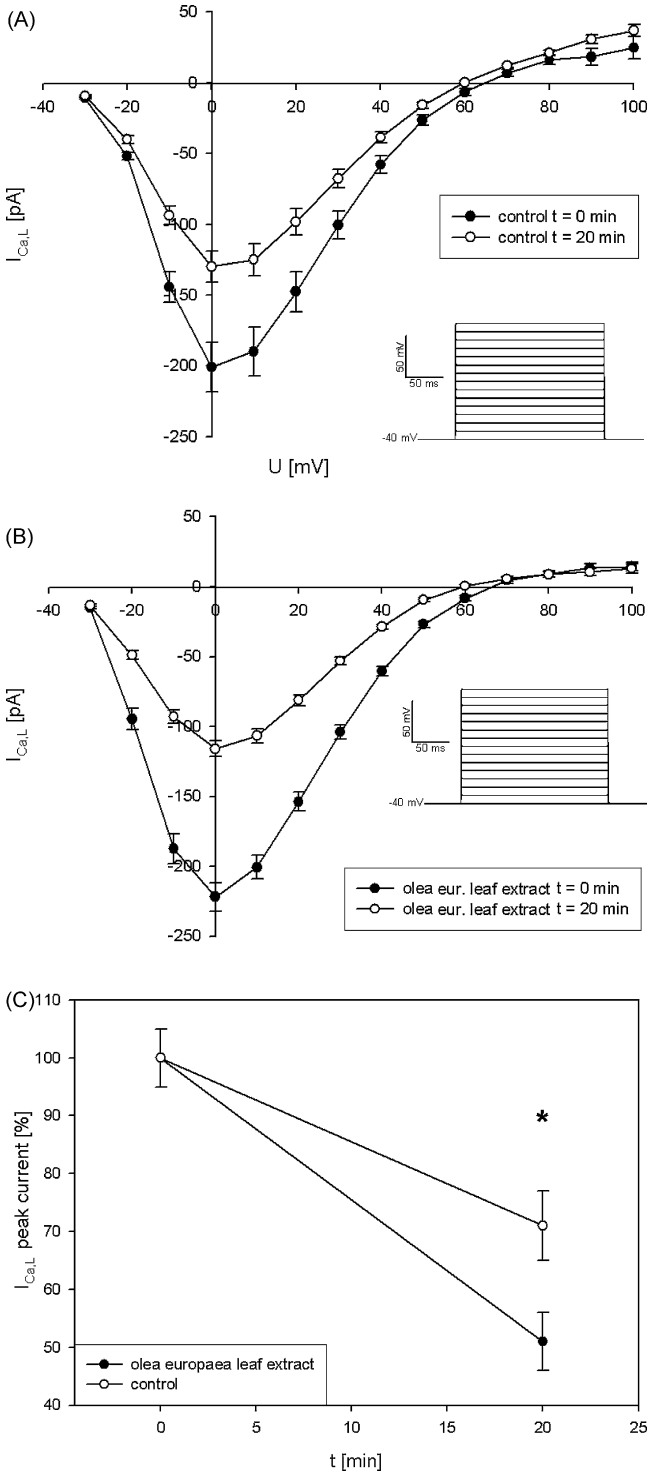

Fig. 4. (A) Current–voltage-relationship of $I_{Ca,L}$ in controls and run down within 20 min. (B) Current–voltage-relationship of $I_{Ca,L}$ in addition of *Olea europaea* leaf extract containing a final concentration of 20 μmol oleuropein. (C) Decrease in L-type calcium channel maximum currents (as percentage of $t_{0\,min}$) in controls and with addition of *Olea europaea* leaf extract containing a final concentration of 20 μmol oleuropein within 20 min. *The difference in the decrease of values is statistically significant.

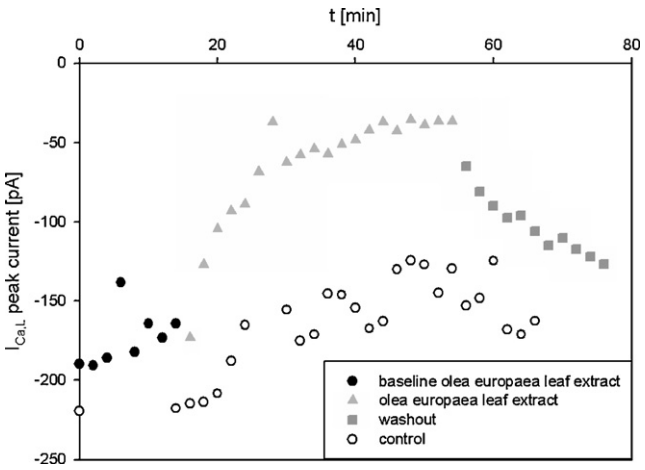

Fig. 5. Example of the time-course of $I_{Ca,L}$ peak current during control experiment and in addition of *Olea europaea* leaf extract.

Olea europaea leaf extract at a final concentration of 20 μM oleuropein for all observed effects being seen at this concentration.

3.2. Voltage clamp

Cardiomyocytes were kept at a holding potential of −40 mV and $I_{Ca,L}$ was elicited by stepwise potential changing from −40 to +100 mV for 200 ms in 10 mV steps. $I_{Ca,L}$ measurement was repeated every 2 min and Fig. 5A shows typical time courses of a control experiment and under *Olea europaea* leaf extract, respectively. Fig. 4 shows the typical current–voltage-relationship for $I_{Ca,L}$ with a maximum current at 0–10 mV and reversal potential at 50–60 mV.

In control experiments ($n = 5$) there was a rundown of $I_{Ca,L}$ with a significant reduction in maximum $I_{Ca,L}$ to 71% within 20 min ($t_{0\,min}$ −166.44 ± 41.88 nS vs. $t_{20\,min}$ −125.0 ± 37.49 nS, $t_{0\,min}/t_{20\,min} = 0.71$) (Figs. 4A and 5). Under *Olea europaea* leaf extract

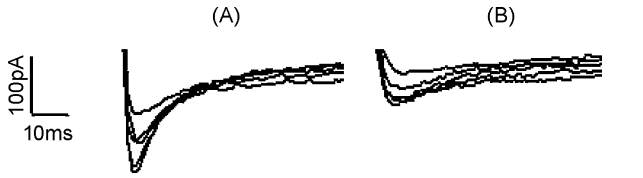

Fig. 6. Exemplary current traces (for purposes of clarity only voltage steps −40/−20 to −40/20 mV are shown): (A) control and (B) under treatment with *Olea europaea* leaf extract.

($n = 9$) this reduction was significantly enhanced and $I_{Ca,L}$ was decreased to 51% in maximum current ($t_{0\,min}$ −231.01 ± 28.85 nS vs. $t_{20\,min}$ −120.32 ± 18.33 nS, $t_{0\,min}/t_{20\,min} = 0.51$) (Figs. 4B and 5). *Olea europaea* leaf extract blocking effects on $I_{Ca,L}$ show a clear onset and the blocking increases during the wash-in phase, during which the *Olea europaea* leaf extract concentration in the experiment chamber increases. When *Olea europaea* leaf extract was washed out there was an increase in $I_{Ca,L}$ again and peak currents reached a level which is comparable to those under control conditions at the same time points. Corresponding exemplary traces are shown in Fig. 6.

In none of our experiments did we observe a change in reversal potential by rundown or *Olea europaea* leaf extract.

Comparing the decreasing $I_{Ca,L}$ in both groups we observed a statistically significant decrease in $I_{Ca,L}$ peak currents of 28% by *Olea europaea* leaf extract containing a final concentration of 20 μmol oleuropein (Fig. 4C).

3.3. Extract characterization

Dry extract was analyzed according to PhEur 5 monograph. The analysis results were in full agreement to PhEur 5 monograph (Kampa, 2006). In addition, standardization was extended in our laboratory by gradient elution HPLC determining six characteristic polyphenols (Fig. 7): oleuropein (13.4%), hydroxytyrosol (0.09%), tyrosol (0.058%), verbascoside (1.05%), apigenin-7-*O*-glucoside (0.15%), luteolin-7-*O*-glucoside (0.4%).

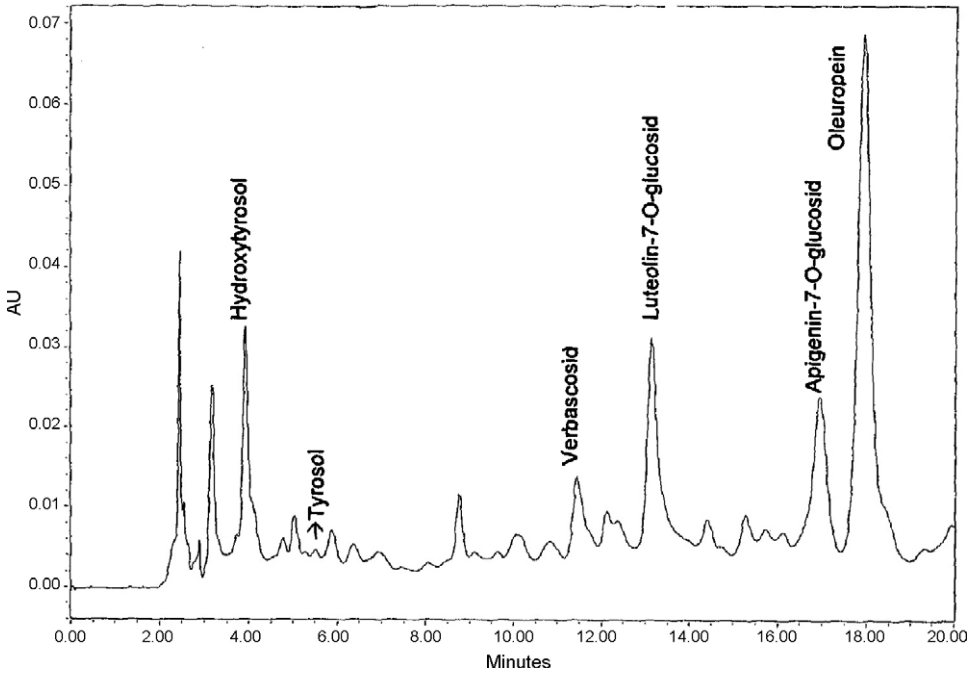

Fig. 7. HPLC separation and determination of six characteristic polyphenols from *Olea europaea* leaves.

4. Discussion and conclusions

We investigated the effects of *Olea europaea* leaf extract on isolated rabbit hearts perfused according to the Langendorff technique as well as on L-type calcium channels in cultured neonatal rat cardiomyocytes. We conclude from the results of our experiments that *Olea europaea* leaf extract suppresses the L-type calcium channel directly and reversibly.

Reduction of LVP, HR and rCF as well as a slight AV-delay are indicators for *Olea europaea* leaf extract exhibiting the typical profile of an antagonist at L-type calcium channel ($I_{Ca,L}$) (Dhein et al., 1993; Nattel, 1993). There seems to be neither an effect on potassium channels (I_K), because ARI and DISP have not been affected, nor an effect on sodium channels (I_{Na}), because there were no changes in TAT and PTP (Dhein et al., 1993).

We tested our assumption of *Olea europaea* leaf extract possibly acting as an antagonist on $I_{Ca,L}$ by voltage clamp experiments and we found indeed that olive leaf extract suppresses the L-type calcium channel $I_{Ca,L}$ directly and reversibly in cultured neonatal rat cardiomyocytes.

The observed effects of *Olea europaea* leaf extract can likely be attributed to the main component oleuropein as other groups have shown a hypotensive effect of oleuropein (Petkov and Manolov, 1972). Besides this Rauwald et al. have found 3,4-dihydroxyphenylethanol, i.e., hydroxytyrosol, the esterifying alcoholic component of the iridoid glycoside oleuropein, exerting vasodilative effects on isolated aortic rings, which have been interpreted as possible calcium antagonistic effects (Rauwald et al., 1991, 1994), which would be in very good agreement to our present data. However, other components, yet unknown, in the extract may also contribute to the effect. Besides the main component oleuropein we have also identified hydroxytyrosol, tyrosol, verbascoside, apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside (Kampa, 2006).

Our results are consistent with those of other investigators. Kosak and Stern described the hypotensive effects of olive leafs and concluded from their experiments that they act as peripheral vasodilators (Kosak and Stern, 1960). Gilani et al. had found indications for the hypotensive effect being mediated via calcium channel blockade as they had excluded effects on muscarinic receptors, had found inhibition of K^+ and phenylephrine-induced contractions of isolated aortic rings and a dose-dependent rightward shift in the Ca^{2+} dose–response curve (Gilani et al., 2005). However they only gained indirect evidence for the molecular mechanism of action as they did not perform voltage clamp experiments. Moreover they used an aqueous-methanolic extract from fresh fruits instead from leafs. Nevertheless oleuropein is contained in leafs as well as in fruits or their oil (Silva et al., 2006) and they are all considered to have beneficial effects on health.

Given that L-type calcium channels play a direct role in cardiovascular function, our findings might help to understand the long-observed beneficial effects of *Olea europaea* leaf extract in treating cardiovascular disease. Investigations of the pharmacology of natural products are necessary to gain evidence concerning activity or lack of activity of medicinal plants used in phytotherapy. Therefore, our experiments contribute to our knowledge of the pharmacology of natural products.

Furthermore *Olea europaea* leaf extract or synthetically derived substances could provide a basis for the development of new therapeutic drugs in the treatment of hypertension and cardiovascular disease. A profile with vasodilatation, heart rate slowing, negative inotropy and slight AV-delay, but lacking effects on action potential-propagation (as indicated by TAT) or duration (as assessed by ARI) might be interesting in coronary heart disease or hypertension (Opie, 1996).

In addition, *Olea europaea* leaf extract does not seem to exert any proarrhythmic effect in isolated rabbit hearts besides induction of bradycardia. The parameters VEC and BTP characterize the activation pattern and sequence. Decreasing values indicate progressive deviation from the initial activation pattern. Therefore, loss of VEC and BTP similarity would indicate alteration of the primary epicardial excitation pattern which is considered to represent an equivalent to proarrhythmic activity. There was a reduction in VEC and BTP similarity. These changes were significant versus time-matched controls at 10 μM oleuropein in BTP and 50 μM oleuropein in VEC, respectively. Therefore, we cannot strictly exclude a proarrhythmic potential of *Olea europaea* leaf extract. Nevertheless these reductions of BTP and VEC similarity were not below the critical values of 50% (BTP) and 10% (VEC), respectively (Fig. 3). There was a major difference between our observations and the proarrhythmic effects of class I antiarrhythmic agents which were described by Dhein and his colleagues in 1993 where VEC and BTP similarity in some cases (e.g., flecainide, propafenone) were reduced to critical values below 10 and 50% where it is merely a matter of time until arrhythmia occur (Dhein et al., 1993).

Since we only investigated direct effects of *Olea europaea* leaf extract on the heart and cardiomyocytes, further investigations were need to answer the questions whether the effect is the same in oral application of *Olea europaea* leaf extract and how many extract is needed therefore. Furthermore effects on other organs and organ systems should be investigated.

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