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Effects of oleic acid on the high threshold barium current in seabass *Dicentrarchus labrax* ventricular myocytes

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Summary

The present study employed a patch clamp technique in isolated seabass ventricular myocytes to investigate the hypothesis that oleic acid (OA), a mono-unsaturated fatty acid, can exert direct effects upon whole-cell barium currents. Acute application of free OA caused a dose-dependent depression of the whole-cell barium current that was evoked by a voltage step to 0 mV from a holding potential of –80 mV. The derived 50% inhibitory concentration (IC_{50}) was 12.49±0.27 μmol l^{-1}. At a concentration of 30 μmol l^{-1}, OA significantly reduced the current density to about 45% of control values, but did not modify either the shape of the current-density voltage relationship or the apparent reversal potential. In addition, OA did not modify the voltage dependence of either steady state inactivation or activation curves. Taken together, these results indicate that physiological concentrations of free OA decrease the conductance of the L-type inward current, without altering its properties of selectivity and its voltage dependence. The inhibitory effect of OA upon the L-type calcium channel may translate, *in vivo*, into a protective effect against arrhythmias induced by Ca^{2+} overload.

Key words: oleic acid, L-type calcium channel, ventricular myocyte, sea bass, fatty acid.

Introduction

The contraction of cardiac myocytes is initiated and graded by an increase in the concentration of free intracellular calcium (Ca^{2+}). In vertebrate hearts, the contractile Ca^{2+} is derived from two sources; external Ca^{2+} influx through the sarcolemma, *via* either Ca^{2+} channels or a Na^+/Ca^{2+} exchanger, and internal Ca^{2+} mobilisation from stores in the sarcoplasmic reticulum. The relative importance of these two sources differs amongst vertebrate groups. In cardiomyocytes from lower vertebrates, such as fish and amphibians, Ca^{2+} influx through the L-type Ca^{2+} current ($I_{Ca,L}$) accounts for the major part of the Ca^{2+} that activates contraction (Vornanen, 1997; Fischmeister and Horackova, 1983; Morad et al., 1981; Coyne et al., 2000; Thomas et al., 1996). This critical importance of $I_{Ca,L}$ for the contraction of fish cardiac myocytes was demonstrated by a profound (>80%) inhibition of force production by verapamil, a blocker of $I_{Ca,L}$, in rainbow trout *Oncorhynchus mykiss* atrial and ventricular myocardium (Aho and Vornanen, 1999). In addition, ryanodine, a blocker of Ca^{2+} release from the sarcoplasmic reticulum, did not reduce contractile force in ventricular strips from a number of ectotherms at physiological temperatures and at stimulation frequencies above 0.2 Hz (Driedzic and Gesser, 1988).

Although intracellular Ca^{2+} is essential for contractions, cell Ca^{2+} overload can lead to cardiac arrhythmias. Indeed, in mammals, an increased intracellular Ca^{2+} concentration activates a transient inward current composed by the Na^+/Ca^{2+} exchanger (Verkerk et al., 2001), a Ca^{2+}-activated chloride current (Verkerk et al., 2000) and a non-selective cation current (Guinamard et al., 2004). If large enough, these can generate sufficient inward current and depolarisation to initiate delayed after depolarizations and arrhythmias.

Fatty acids have been shown to have multi-faceted effects upon the cardiac physiology of higher vertebrates (mammals), and this has been the focus of much study (Sergiel et al., 1998; Pepe and McLennan, 2002; Nair et al., 1997). The heart depends heavily upon a supply of FA provided in the bloodstream, for use as aerobic fuels and also as membrane components. In mammals, FA moieties are present in blood either as unesterified molecules (free FA), or in an esterified form incorporated into mono-, di-, and triacylglycerols, phospholipids and cholesteryl esters (Van Der Vusse et al.,

1992). Various unesterified FA (NEFA) have in fact been shown to influence Ca^{2+} homeostasis in ventricular cells. For example, the n-3 poly-unsaturated fatty acids (n-3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can induce a significant inhibition of $I_{Ca,L}$ in mammal ventricular myocytes (Xiao et al., 1997; Pepe et al., 1994; Hallaq et al., 1992; Ferrier et al., 2002). The same n-3 PUFA can decrease cardiac sarcoplasmic reticulum Ca^{2+} release (Negretti et al., 2000; Swan et al., 2003; Honen et al., 2003). Those effects of n-3 PUFA are known to protect hearts against arrhythmia induced by Ca^{2+} overload. Aside from these established effects of n-3 PUFA, fatty acids such as arachidonic acid (AA) and oleic acid (OA) have also been reported to modulate $I_{Ca,L}$, both positively and negatively (Shimada and Somlyo, 1992; Liu et al., 2001; Xiao et al., 1997; Huang et al., 1992).

The potential effects of NEFA on fish cardiac myocytes have never been studied. However, in a previous study, we found that high tissue levels of OA in sea bass significantly improved cardiac performance, in particular cardiac scope for work (Chatelier et al., 2006). Oleic acid is a major component of blood NEFA in fish and plasma concentrations can reach 600 μmol l^{-1} in female sockeye salmon *Oncorhynchus nerka* (Ballantyne et al., 1996). The aim of the present study was to investigate the hypothesis that unesterified OA has direct effects upon calcium transport in sea bass ventricular myocytes. To this end, the whole-cell configuration of the patch-clamp technique was employed to investigate the electrophysiological properties of the L-type Ca^{2+} channel.

Materials and methods

Animals

European sea bass *Dicentrarchus labrax* L., mass 266±18 g (mean ± s.e.m.), were obtained from a commercial supplier on the Ile de Ré (Charente Maritime, France). They were maintained in 1 m^2 fibreglass tanks (water volume approximately 400 l) provided with biofiltered seawater (SW) at a salinity of 30‰ and a temperature of 20°C. Fish were subjected to a natural photoperiod and fed with commercial fish food daily. They were acclimated to these conditions for one month prior to the experiments.

Ventricular cardiomyocyte isolation

Bass were anaesthetized with tricaine methane sulphonate (MS-222) at a concentration of 0.1 g l^{-1} and their heart rapidly excised. Single ventricular cells were obtained by enzymatic dissociation using a protocol derived from that described elsewhere (Vornanen, 1997). Briefly, a cannula was inserted through the bulbus arteriosus into the ventricle and hearts rinsed for 2 min with a control solution (in mmol l^{-1}): NaCl 130, CsCl 5.4, NaH_2PO_4 0.04, $MgSO_4$ 2.5, $CaCl_2$ 1.8, glucose 10, Hepes 10 (pH 7.6). Following the rinsing, hearts were perfused with a Ca^{2+}-free solution, to disrupt Ca^{2+}-dependent cellular bonds. The Ca^{2+}-free solution contained (in mmol l^{-1}): NaCl 100, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 6.7, taurine 50, glucose 20, Hepes 10, EGTA 0.1 (pH 7.1). Hearts were then perfused for 15 min with the Ca^{2+}-free solution complemented with collagenase (type IA, 0.36 mg ml^{-1}), trypsin (type III, 0.24 mg ml^{-1}) and BSA (100 mmol l^{-1}). Following enzymatic treatment, the ventricle was cut into small pieces and dissociated with a Pasteur pipette in the calcium-free solution. Ca^{2+} was then slowly increased by adding the control solution progressively. Cells were kept in the control solution at 20°C and used for patch-clamp experiments within 6 h. All chemicals were purchased from Sigma-Aldrich (France).

Recording of L-type barium current ($I_{Ba,L}$)

The whole-cell patch-clamp technique was used to study the effects of OA on the electrophysiological properties of the L-type Ca^{2+} channel. A large quantity of isolated cells (>50%) were calcium tolerant. Only calcium-tolerant myocytes with clear striations (capacitance=41.04±1.06 pF; N=38) were used for experiments. Voltage clamp experiments were performed using an Axopatch 200B amplifier with a CV 203BU headstage (Axon instrument, CA, USA). Pipettes were pulled from borosilicate glass capillaries (Clark electromedical instrument, Pangbourne, UK) using a model P-97 Flamming/Brown micropipette puller (Sutter Instrument Company, Novato, CA, USA) and had a resistance of 2–4 MΩ when filled with pipette solution. Junction potentials were zeroed prior to seal formation. Currents were filtered at 2 kHz and analyzed using PClamp 9 software.

Current recordings were made from the same myocyte before, during, and after exposure to OA. During experiments, various concentrations of OA were rapidly applied to the solution perfusing the cell, by means of a microperfusion device (Microdata Instrument, South Plainfied, NJ, USA). Myocytes were perfused at a rate of ~300 μl min^{-1}. Oleic acid was dissolved in 95% ethanol at a concentration of 30 mmol l^{-1} and stored under a nitrogen atmosphere at –20°C before use. The experimental concentrations of the FA were obtained by dilution of the stock, and contained negligible ethanol (lower than 0.1%). The pipette solution for recording $I_{Ba,L}$ contained (in mmol l^{-1}): CsCl 130, $MgCl_2$ 1, oxaloacetate 5, succinate 5, MgATP 5, TEA-Cl 15, EGTA 5, Hepes 10 (pH 7.2). The bathing and control perfusion solutions contained (in mmol l^{-1}): NaCl 130, CsCl 5.4, $MgCl_2$ 1, $BaCl_2$ 5, glucose 10, Hepes 10, tetrodotoxin (TTX) 0.001 (pH 7.6). As reported in other fish species (Nurmi and Vornanen, 2002; Shiels et al., 2004), TTX at 1 μmol l^{-1} completely abolished the fast sodium current in seabass cardiomyocytes (not shown). Caesium was added to inhibit potassium currents. The use of Ba^{2+} as the charge carrier instead of Ca^{2+} has a number of advantages: (1) conductance for Ba^{2+} ions *versus* Ca^{2+} ions through calcium channels is larger (Hess et al., 1986), thereby increasing the signal-to-noise ratio; (2) in the presence of Ba^{2+} ions, the inactivation of L-type Ca^{2+} channel is slowed while the inactivation of the T-type is unaffected, which helps for their identification (Bean, 1985); (3) it reinforces blocks to many K^+ currents; and (4) because of the small size of seabass ventricular myocytes (≈40 pS), rundown of the current is

sometimes prominent and the use of Ba^{2+} attenuates this problem. Furthermore, when experiments were performed with external calcium solutions ($[Ca^{2+}]$=5 mmol l^{-1}), many cells exhibited a calcium-activated component, most probably due to the activation of both calcium-activated chloride currents (Zygmunt and Gibbons, 1992) and the calcium-activated non-specific cation current (Guinamard et al., 2004). These components were suppressed in external Ba^{2+} conditions.

To control for any potential effects of ethanol, control perfusates contained the same proportion of ethanol as the OA perfusates (below 0.1%). This concentration of ethanol had no discernible effect on Ba^{2+} currents (not shown).

Data analysis

Under voltage clamp conditions, cells were held at –80 mV and stepped in 10 mV increments for 300 ms from –60 mV to 50 mV. Current density, pA/pF, of $I_{Ba,L}$ of single ventricular myocytes was calculated by divided the amplitude of the peak current by the cell membrane capacitance. The relative current of $I_{Ba,L}$ after the treatment of OA was calculated as $I_{(OA)}/I_{(control)}$ from the same cell.

The steady-state activation curves were estimated from the relative membrane conductance as a function of potential as $G_{Ba}=I_{Ba}/(V_m-V_{rev})$ where G_{Ba} is the peak conductance, I_{Ba} the peak of barium current for the test potential V_m, and V_{rev} the apparent reversal potential of the barium current. The steady state inactivation curves were obtained using a two-pulse protocol. A 1.2 s depolarizing conditioning pulse to different voltages from –90 mV to 60 mV (increments of 10 mV) was followed by a 300 ms test pulse to the voltage at which the maximal L-type barium current were obtained (0 mV). Conditioning and test pulse were separated by a 10 ms return to the holding potential (–90 mV). The activation, inactivation and conductance data were fitted with the simple Boltzmann function ($I/I_{max}=\{1+\exp[(V_m-V_{0.5})/K]\}^{-1}$). The dose–response relationships of OA suppression of $I_{Ba,L}$ was fitted to the Hill equation $f(x)=A/[1+(IC_{50}/x)^h]$, where A is maximal effect, IC_{50} is the OA concentration required to give half of the maximal effect and h is the Hill factor. All experiments were performed at a room temperature of 20–23°C.

Statistical analysis was performed with either Student's t-test or analysis of variance (ANOVA). Values of $P<0.05$ were considered significant. Statistical data were given as mean ± s.e.m.

Results

Fig. 1A shows an example of a typical barium current evoked, in a single seabass ventricular myocyte, by a step in membrane potential from a holding potential of –80 mV to 0 mV, and the effect of 30 μmol l^{-1} OA on this current. In this cell, 30 μmol l^{-1} OA applied to the extracellular solution decreased the peak amplitude by 31.42% after approximately 5 min of perfusion, without causing a change in time to peak. At this concentration, OA had not affect on the inactivation time course (τ) (τ was estimated at 106.03±29.23 in control *vs* 86.56±12.83 with 30 μmol l^{-1} OA, N=10, $P>0.05$); but depressed the charge carried (Q_{Ba} was estimated at 1.75±0.22 coulomb/farad in control *vs* 0.79±0.18 coulomb/farad with 30 μmol l^{-1} OA; N=11 and 10, $P<0.01$). The residual inward current elicited from –80 to 0 mV was completely abolished after addition of 3 μmol l^{-1} nifedipine (N=3), a dihydropyridine Ca^{2+} channel antagonist (Fig. 1B). Under these experimental conditions, the absence of the insensitive nifedipine inward current indicated that the barium current was not carried by T-type Ca^{2+} channels. Contrary to the L-type Ca^{2+} channels, dihydropyridine calcium-channel blockers do not affect the T-type Ca^{2+} channels of cardiac myocytes (for a review, see Bean, 1989).

Fig. 1C illustrates the time course of OA-induced suppressant effects on the $I_{Ba,L}$ peak amplitude elicited by clamp from –80 to 0 mV, for four different OA concentrations.

In the absence of OA (control), the current increased transiently during the first 2 min and slowly decreased by 8.63±5.65% (N=4) at 6 min. This weak reduction, which was not statistically significant, indicated that the 'run-down' process of the inward Ca^{2+} current previously reported in mammalian myocytes is slow in the bass cardiomyocytes. In a similar manner, OA at low concentrations (5 μmol l^{-1}) did not have a marked inhibitory effect on the amplitude after 5 and 6 min of perfusion. However, the application OA at 10, 30 and 100 μmol l^{-1} for 5 min induced a significant reduction in $I_{Ba,L}$. Interestingly, the more concentrated was the OA, the earlier the significant reduction in $I_{Ba,L}$ appeared. Indeed, 100, 30 and 10 μmol l^{-1} OA induced a significant reduction of $I_{Ba,L}$ peak amplitude after 1, 3 and 5 min of OA application, respectively.

Fig. 1D shows the concentration-dependent curve of suppression of $I_{Ba,L}$ by OA, at 5 min of perfusion. The decrease in $I_{Ba,L}$ was 7.39±6.48 (N=3), 19.42±5.79 (N=4), 42.52±7.15 (N=10) and 48.96±5.22% (N=6) for OA at 5, 10, 30 and 100 μmol l^{-1}, respectively. The reduction in $I_{Ba,L}$ was significantly higher ($P<0.05$) for OA at 100 μmol l^{-1} and 30 μmol l^{-1} than for OA at 5 μmol l^{-1} or 10 μmol l^{-1}. Note that even at the highest concentrations of OA, the inward current was not fully blocked. Fitting the dose–response curve with the Hill equation yielded a half-effect concentration (IC_{50}) of 12.49±0.27 μmol l^{-1} and a Hill factor of 1.97±0.07. Fig. 2A illustrates the current–voltage relationships for $I_{Ba,L}$ in the absence of OA or after 5 min of 30 μmol l^{-1} OA perfusion. The amplitude of the peak current was normalized by cell capacitance and was plotted as a function of voltage. The whole cell inward current exhibited $I_{Ba,L}$ properties with a threshold potential at about –40 mV and a maximum at 0 mV. Under control conditions, maximum current density was 11.5±0.95 pA/pF. The application of 30 μmol l^{-1} OA significantly reduced $I_{Ba,L}$ density, to about 45% of its control level ($P<0.01$). However, the shape of the current–voltage relations was unaffected by OA; there was no shift in activation or maximal peak current potential. The apparent reversal potential estimated by extrapolation from potentials between 0 and 30 mV was not significantly different between control and 30 μmol l^{-1} OA, being 35.64±1.33 mV and 31.78±3.37 mV,

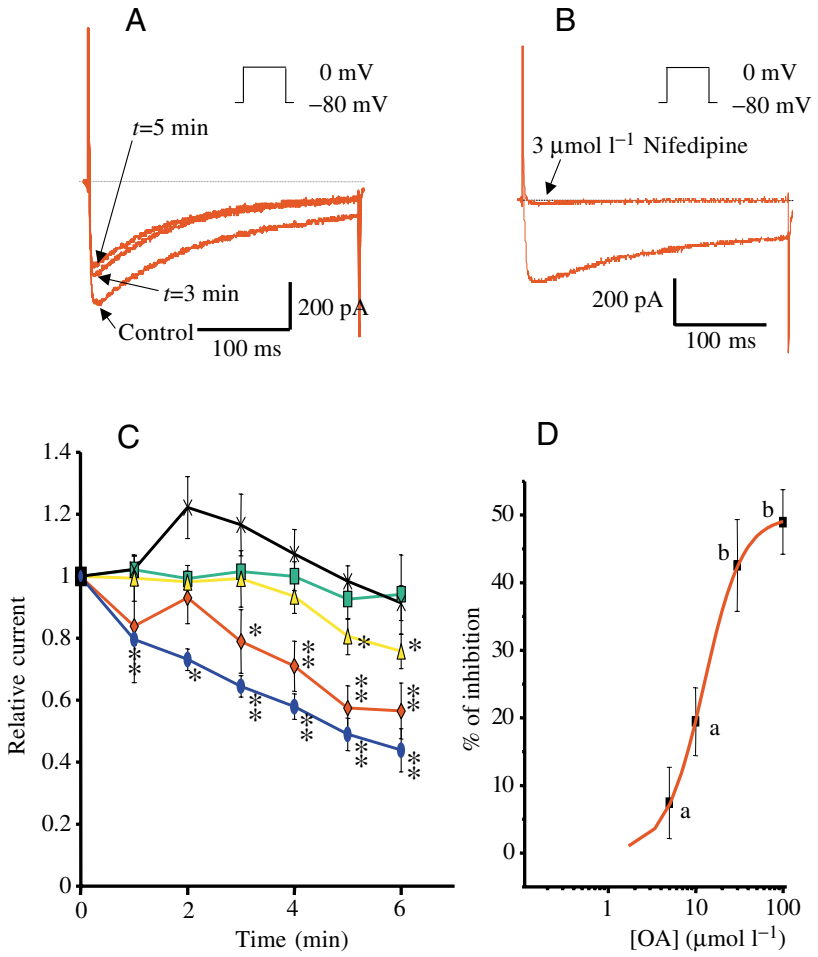

Fig. 1. Effect of OA on the voltage-activated L-type current carried by barium in sea bass ventricular myocytes. (A,B) The current was elicited by a step in membrane potential to 0 mV from a holding potential of –80 mV (insert) during 300 ms (A) at 1/20 Hz. Superimposed traces show responses of a cell in control solution, after 5 min exposure to 30 µmol l^{-1} OA (A) and 3 µmol l^{-1} nifedipine (B) (capacitance=40.71 pF). (C) Time course of the inhibitory effect of OA on the peak $I_{Ba,L}$. Peak currents were normalised to their initial peak current recorded at t=0 min of perfusion and are expressed as mean ± s.e.m. Black crosses, control perfusion without OA (N=11); green squares, yellow triangles, red diamonds and blue circles, OA concentrations of 5 (N=3), 10 (N=4), 30 (N=10) and 100 µmol l^{-1} (N=6), respectively. Significant differences between control and the different OA concentrations are indicated (*P<0.05, **P<0.01). (D) Concentration dependence of inhibition by OA on $I_{Ba,L}$ after 5 min of perfusion. The number of cells treated with OA at 5, 10, 30 and 100 µmol l^{-1} is 3, 4, 10 and 6, respectively. The line was obtained by fitting data to the Hill equation (see Materials and methods), which gave an IC_{50} of 12.49±0.27 µmol l^{-1} and a Hill factor of 1.97±0.07. Values are mean ± s.e.m. Dissimilar letters indicated a significant difference (P<0.05) of $I_{Ba,L}$ diminution between the different OA concentrations.

respectively, indicating that the FA did not alter the selectivity properties of the current. The reversibility of OA was tested on three cells (not shown). After 3 min of perfusion with the control solution, the effect of OA was not completely reversed.

Fig. 2B shows the conductance (see Materials and methods) divided by membrane capacitance (nS/pF), plotted against the membrane potential. The curves, which have been constructed from the current–voltage relationships shown in Fig. 2A (see Materials and methods), revealed that the conductance decreased significantly (P<0.05) with 30 µmol l^{-1} OA at voltages greater than –20 mV, when compared with the control. Maximum conductance was significantly lower in the presence of

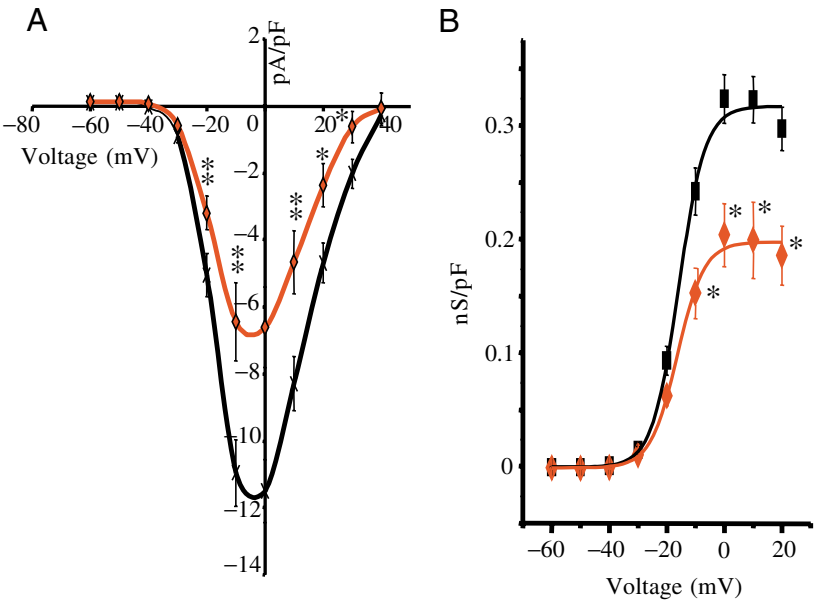

Fig. 2. Effect of OA on density–voltage and conductance–voltage relationships. (A) Current density–voltage relationships were plotted for the L-type inward current of sea bass ventricular myocytes in controls (black crosses; N=11 between –60 to +30) and in the presence of 30 µmol l^{-1} OA (red diamonds; N=9 between –60 to +30). For +40 mV N=9 and N=7 in control and OA conditions, respectively. (B) Conductance curves constructed from the density–voltage curves in A. Values are mean ± s.e.m. Significant differences between control and the different OA concentrations are indicated (*P<0.05, **P<0.01).

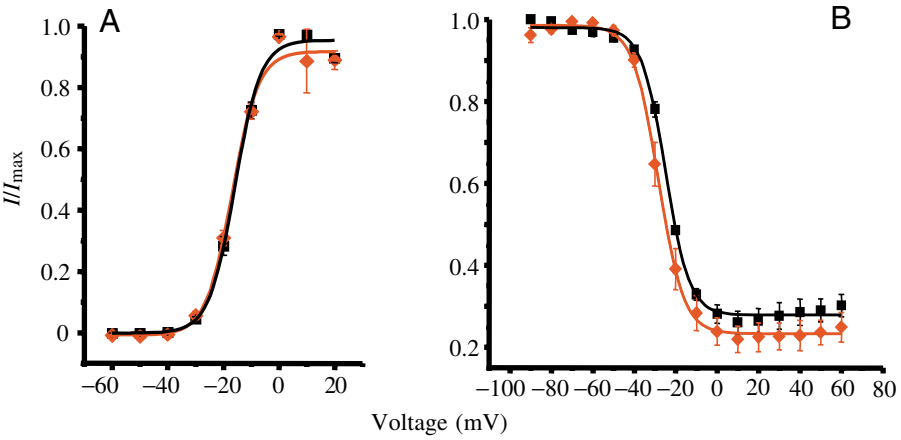

Fig. 3. Steady-state activation (A) and inactivation (B) curves of calcium channels in control and after 5 min of perfusion with 30 μmol l^{-1} OA. Current amplitude were normalised to maximum current and plotted against holding potential. Data were fit using the appropriate form of the Boltzmann equation (see Materials and methods). Individual ventricular myocytes used for inactivation and activation curves were respectively three and 11 for control and four and nine for 30 μmol l^{-1} OA.

30 μmol l^{-1} OA (0.20±0.03 nS/pF; N=9) than in control conditions (0.32±0.02 nS/pF; N=11).

Fig. 3 compares the steady state inactivation and activation curves of Ca^{2+} channels in controls and after a 5 min application of 30 μmol l^{-1} OA. Curve-fitting (see Materials and methods) indicated that there was no significant difference between the control and 30 μmol l^{-1} OA midpoint potentials ($V_{1/2}$) for inactivation (–25.99±1.23 mV and –28.06±1.55 mV, respectively). In a similar manner, midpoint potentials for activation were not significantly different between control and 30 μmol l^{-1} OA (–15.74±0.82 mV and 16.23±0.71 mV, respectively). Additionally, no significant difference between control and 30 μmol l^{-1} OA was observed between maximum inactivation or maximum activation. Inactivation and activation slope parameters (K) were not significantly different between control (5.74±0.32 and 4.76±0.26, respectively) and in the presence of 30 μmol l^{-1} OA (6.01±0.71 and 4.99±0.35, respectively). Steady-state inactivation began around –50 mV, with maximum inactivation reached around 10 mV. Steady-state activation began at voltages greater than –40 mV, with a peak around 10 mV. The superposition of voltage-dependence for the activation and inactivation of inward currents revealed that there was a voltage range within which the activation and inactivation curves overlapped. This overlap was estimated between –40 and 0 mV and maximal between –16 and –18 mV.

Discussion

The present study is the first to investigate potential effects of NEFA on ion transport by fish cardiac myocytes. The data reveal that unesterified OA exerts a direct suppressant effect upon barium currents through calcium channels. The suppression of the whole-cell Ba^{2+} currents evoked by a voltage step to 0 mV from holding potential of –80 mV was dose dependent, with an IC_{50} value of 12.49±0.27 μmol l^{-1}. At a NEFA concentration of 30 μmol l^{-1}, OA significantly reduced the current density to 42.52±7.15% without altering either the shape of the current-density voltage relationships or the apparent reversal potential. Although we have no information on the concentration of unesterified OA in the fish cardiac cell environment, we used the concentrations that are typically used for similar studies on mammals (Xiao et al., 1997; Leifert et al., 1999; Huang et al., 1992).

The effects of OA on the seabass cardiac myocytes are consistent with the findings of Shimada and Somlyo (Shimada and Somlyo, 1992), who demonstrated that unesterified OA can induce a decrease in $I_{Ba,L}$ in rabbit intestinal smooth muscle. Xiao et al. (Xiao et al., 1997) reported that 5 μmol l^{-1} OA had no effect on $I_{Ca,L}$ in neonatal rat cardiomyocytes. This result is not, however, incompatible with our findings, whereby the effect of OA was concentration dependent and only became significant above concentrations of 10 μmol l^{-1}. Nevertheless, these results differ from those reported by Huang et al. (Huang et al., 1992) in guinea pig ventricular myocytes. In that myocyte preparation, 3 μmol l^{-1} OA induced a large transient increase in $I_{Ca,L}$, the low Ca^{2+} inward current began to increase at 2 min, reached a plateau in 14 min, and then started to decrease after 25 min of perfusion. There are a number of studies showing that unesterified n-3 PUFA, specifically EPA and DHA, can modulate L-type Ca^{2+} channels in mammalian hearts (Xiao et al., 1997; Pepe et al., 1994; Hallaq et al., 1992; Ferrier et al., 2002). Concentrations of 5 μmol l^{-1} induced a large and significant decrease in $I_{Ca,L}$ of 83% and 62% for EPA and DHA, respectively (Xiao et al., 1997). Our study shows that OA has similar effect but of a lesser magnitude than EPA and DHA, with a maximum inhibition of 49±5% at 100 μmol l^{-1} OA.

At high temperature, rainbow trout hearts become arrhythmic (Heath and Hughes, 1973). Farrell hypothesized (Farrell, 2002) that oxygen supply may become insufficient to meet cardiac oxygen demand at high temperatures, inducing cardiac dysfunction. Work on mammals has demonstrated that when oxygen falls below a critical level in the cytoplasm, it can induce an increase in Ca^{2+} concentration (reviewed by Carmeliet, 1999). This Ca^{2+} excess produces arrhythmogenic transient inward currents (I_{ti}), which depolarize the cell membrane, generating delayed after depolarizations. This is assumed to reflect at least three components, one supported by Na^{+}/Ca^{2+} exchange (Verkerk et al., 2001), a second involving

a $[Ca^{2+}]_i$-activated chloride current (Verkerk et al., 2000), and a third component which is carried by non-selective cation channels (Guinamard et al., 2004).

In our study, OA induced a decrease in ion influx through the sarcolemma *via* L-type Ca^{2+} channels, an effect that could modulate I_{ti}. Therefore, OA may have a protective effect against Ca^{2+} overload and consequent arrhythmias. This is in accord with the study of Mackay and Mochly-Rosen (Mackay and Mochly-Rosen, 2001), where 40 μmol l^{-1} OA appeared to protect cardiac myocytes from prolonged ischemia. It was also observed that OA appears to be able to reduce Ca^{2+} release from the sarcoplasmic reticulum (Honen et al., 2003). In the seabass, it would be interesting to extend the current observations by investigating the effects of OA on free intracellular Ca^{2+}. Moreover, because barium was used as a calcium substitute in the current study, it would be interesting to confirm the effects of OA on calcium-dependent inactivation of calcium channels.

In conclusion, this is the first demonstration that NEFA can have direct effects upon ion transport in fish cardiac myocytes. The suppression of the L-type Ca^{2+} channel achieved with OA in seabass ventricular myocytes was less than that achieved by similar doses of EPA and DHA in mammals (Xiao et al., 1997). The effects of OA may be beneficial for the seabass *in vivo*, protecting against Ca^{2+} overload and consequent arrhythmias induced by myocardial hypoxia. The dietary intake of cardioprotective FA, such as OA, may have beneficial effects when water temperature is elevated or when water oxygen content is low. These situations may frequently be encountered by migrating and foraging seabass, so the impact of dietary FA in such teleost species deserves further study.

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