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The effect of the isomers of cyclo(Trp-Pro) on heart and ion-channel activity

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Abstract

Cyclo(L-Trp-L-Pro) has shown potential for use in the treatment of cardiovascular dysfunction. The aim of the study was to determine the effects of the isomers of cyclo(Trp-Pro) – cyclo(L-Trp-L-Pro), cyclo(L-Trp-D-Pro), cyclo(D-Trp-L-Pro) and cyclo(D-Trp-D-Pro) – on heart and ion-channel activity. The effects on L-type Ca^{2+}-channel, Na^{+}-channel and inward rectifier K^{+}-channel activity were determined by using the whole-cell patch-clamp technique on myocytes of guinea-pig origin. Dependence on the membrane potential in terms of Ca^{2+}-channel activity was also investigated. A modified Langendorff method was used to determine the effects of the isomers on heart rate, coronary flow, duration of ventricular tachycardia and arrhythmia, time to sinus rhythm and QRS interval on the rat isolated heart. Cyclo(L-Trp-L-Pro), cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro), 100 μM, showed agonism towards Ca^{2+}-channel activity, while cyclo(D-Trp-L-Pro) caused a blockage of the current. The action of cyclo(D-Trp-L-Pro) was shown to be independent of membrane potential. No significant effect ($P > 0.05$) on the inward rectifier K^{+} current was observed in the presence of cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro), while antagonism was noted in the presence of cyclo(L-Trp-L-Pro) and cyclo(D-Trp-L-Pro). All isomers showed antagonist effects on the Na^{+} channel. No adverse effects were noted on chronotropic effects in the presence of 200 μM cyclo(L-Trp-L-Pro) and cyclo(D-Trp-D-Pro) ($P > 0.05$), while cyclo(L-Trp-D-Pro) significantly increased the heart rate. Cyclo(D-Trp-L-Pro) significantly reduced the heart rate ($P < 0.05$). In addition, no significant effects were observed on the coronary flow rate in the presence of the isomers. All isomers significantly reduced the duration of ventricular tachycardia and arrhythmia, as well as the time to sinus rhythm. Furthermore, no change in the QRS intervals was noted in the presence of the isomers in comparison with the control, with a significant increase being noted for cyclo(D-Trp-D-Pro) ($P < 0.05$) in reference to the other isomers. The isomers thus show antiarrhythmic potential and may manifest as novel agents in the treatment of cardiovascular dysfunction, since a decrease in ventricular fibrillation may reduce the mortality rates in acute myocardial infarction.

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Introduction

Despite intensive research, ischaemic heart disease remains a serious problem. Death and cardiac arrhythmias are often associated, particularly ventricular fibrillation (VF), which may be initiated by ischaemia and reperfusion. Antiarrhythmic agents devoid of serious side effects are thus needed. Rational treatment of cardiac arrhythmias therefore necessitates an absolute understanding of the pharmacokinetic and pharmacodynamic properties of potential cardiac disease agents (Hashimoto et al 1986). These drugs may be developed rationally by drug design in terms of highly selective cellular action. This, however, needs prior knowledge that the selective action is beneficial in antiarrhythmic activity (Rees & Curtis 1993). Screening of antiarrhythmic agents for anti-VF activity is important since VF is a major cause of death in acute myocardial infarction.

The therapeutic effectiveness of L-type Ca^{2+}-channel antagonists in cardiovascular pathologies is based on the inhibition of Ca^{2+} influx in depolarized smooth muscle (Godfraind & Govoni 1995). Ca^{2+}-channel antagonists are known to exhibit antiarrhythmic activity, have negative inotropic effects, inhibit heart rate and show relative vasodilatory activity.

Disturbance in the intracellular homoestasis – especially Ca^{2+} overload in heart muscle during reperfusion injury – is also the main cause of cell death after coronary infarction. However, in this case, blocking the L-type Ca^{2+} channels does not prevent damage but blocking the fast tetrodotoxin-sensitive Na^+ channels does (Ver Donck & Borgers 1991).

K^+ channels are also the target in the treatment of various disease states such as non-insulin-dependent diabetes mellitus, asthma and cardiac arrhythmias (Sensch et al 2000).

K^+ channel blockers (Class III antiarrhythmic drugs) are useful in the treatment of cardiac arrhythmias (Dupuis & Adamantidis 1995). The cell can be driven into a resting state by repolarizing currents that are generated by the opening of voltage-gated K^+ channels under normal conditions. These drugs prolong the repolarization phase (Godfraind & Govoni 1995). Research in our laboratories has shown the potential of the cyclic dipeptide cyclo(Trp-Pro) as an antimicrobial substance, as well as its potential usage in the treatment of cardiovascular dysfunction (Milne et al 1998). Investigation of the activity of the isomers may result in the formulation of a drug entity with greater activity or specificity than the L-form, or as the case may be, the isomers may show reduced or no activity at all.

In this study, we determined the effect of the isomers on inward rectifier K^+ current and Ca^{2+}-channel and Na^+-channel activity using the whole-cell patch-clamp technique on ventricular myocytes isolated from the guinea-pig. A modified Langendorff method was used to determine the effects of the isomers on heart rate and coronary flow in the isolated, perfused rat heart. The time taken to stop ischaemia-induced arrhythmias and ventricular tachycardia, as well as the time taken for the heart rate to return to normal sinus rhythm was also determined. The ECG was examined to determine the effects of the isomers on the QRS complex.

Materials and Methods

Isomer solutions

The method of Grant et al (1999) was used to synthesize the isomers of cyclo(Trp-Pro). The isomers (M_r = 284) were stored at 4°C until use. All materials and solvents used were of analytical grade. The isomers were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 0.5%, to aid dissolution.

Whole-cell patch-clamp method

Calcium-channel activity

The whole-cell patch-clamp technique (Hamil et al 1981) was performed on excitable, ventricular cells, isolated from guinea-pigs, as described previously by Mitra & Morad (1985) and adjusted by Tytgat (1994). A Dagan (Model 8800 Total clamp) amplifier was used for patch clamping and electrodes were made from borosilicate glass using a Narishige PP 83-model puller. Electrodes (2–4 mΩ) were heat polished before seal formation. Currents were registered using Clampex software (Labmaster TM40, version 5.5.1). To determine the effects of the compounds on Ca^{2+}-channel activity, the cells were exposed to 100 μM solutions (pH 7.4) of the respective isomers. The intracellular solution contained in mM: 125 CsCl, 2 $MgCl_2$, 5 EGTA, 10 HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), 1 $CaCl_2$, 3 Mg-ATP and 10 glucose. The pH was adjusted to 7.2 with NaOH. The extracellular solution contained in mM: 138 Tris, 0.5 $MgCl_2$, 10 HEPES, 5.4 $CaCl_2$, 20 CsCl and 5 glucose.

Inward Ca^{2+} currents were recorded from a holding potential of −90 mV with 100-ms depolarizing steps to test potentials between −50 and 25 mV in 5-mV steps every 6 s. The current–voltage relationships are given as the measured maximum current value at a specific test potential. To determine whether the effect is influenced by the holding potential, inward Ca^{2+} currents were also recorded at a holding potential of −45 mV with depolarizing steps to 5 mV. The maximum current value at 5 mV was compared at a holding potential of −90 and −45 mV. All experiments were recorded at room temperature.

Sodium-channel activity

The whole-cell patch-clamp technique was used to record Na^+ currents under voltage clamp conditions from single cells isolated with enzymatic dispersion from the ventricles of guinea-pig, as described above. The cells were then exposed to 10 μM solutions (pH 7.4) of the respective compounds. The intracellular solution contained in mM: 5 $MgCl_2$, 125 CsCl, 20 TEA-Cl (tetraethylammonium chloride), 10 HEPES, 15 EGTA (ethylene glycol-bis-(2-aminoethylether)*N*,*N*,*N*′,*N*′-tetra-acetic acid) and 5 Na_2-ATP. The pH was adjusted to 7.2 with CsOH. The extracellular solution contained in mM: 20 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 76 Tris and 5 HEPES. Sodium currents were recorded from a holding potential of −90 mV to a test potential of −30 mV to determine if the isomers were active on the Na^+ channel.

Potassium-channel activity

The whole-cell patch-clamp technique was used to record inward rectifier K^+ currents under voltage clamp conditions from single cells isolated with enzymatic dispersion from the ventricles of guinea-pig, as described for the Ca^{2+}-channel activity. The cells were then exposed to 100 μM solutions (pH 7.4) of the respective compounds. The intracellular solution contained in mM: 140 KCl, 2 $MgCl_2$, 11 EGTA, 10 HEPES, 1 $CaCl_2$ and 5 Na_2-ATP. The pH was adjusted to 7.2 with KOH. The extracellular solution contained in mM: 130 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES-NaOH, 1.8 $CaCl_2$ and 10 glucose. Currents were recorded during 500-ms hyperpolarizing steps from a holding potential of −80 mV to test potentials between −140 mV and −50 mV.

Isolated heart perfusion

A modified Langendorff method was used to study the effects of the isomers on heart rate, coronary flow and reperfusion-induced arrhythmias in rat hearts (Langen-

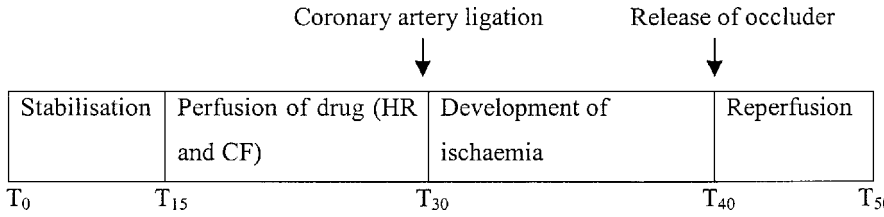

Figure 1 The experimental protocol for the rat isolated heart perfusion. T, time (min); HR, heart rate; CF, coronary flow.

dorff, 1895). Male Long Evans rats, 250–350 g, were placed under light ether anaesthesia to a loss of blink and pain reflexes. Once removed, the hearts were arrested in ice-cold Krebs Henseleit bicarbonate buffer (KHBB) (pH 7.4). KHBB contained in mM: 118 NaCl, 2.5 $NaHCO_3$, 4.75 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4 \cdot 7H_2O$ and 2.5 $CaCl_2 \cdot 2H_2O$. The heart was perfused via an aortic cannula. Occlusion was achieved by using a silk suture (Clinisut, South Africa) and a rigid polyvinyl occluder (5 mm × 1 mm). Successful occlusions were characterized by a 50% decrease in coronary flow rate. Reperfusion of the infarcted area of the left ventricle occurred once the tension on the occluder was removed. Acceptable reperfusion was characterized by a 90% increase in coronary flow rate.

After a 15-min stabilization period, the isomer solution (200 μM isomer dissolved in DMSO to a final concentration of 0.5%) was perfused into the heart for 15 min. During this period, the heart rate and coronary flow were monitored every 5 min. Following this, the perfusion buffer was changed to a KHBB containing a lower concentration of K^+ (3.3 mM). At the same time, the left descending coronary artery was occluded for a period of 10 min, allowing the development of ischaemia. The occluder was then released and the heart reperfused with the low-K^+ KHBB and respective isomer. Control samples were perfused with KHBB containing 0.5% DMSO.

A summary of the experimental protocol is set out in Figure 1.

Measurement of ventricular tachycardia and arrhythmia, time to sinus rhythm and QRS interval

ECGs recorded on PolyView Data Acquisition and Analysis System (Version 2.0, 1997) were examined for duration of ventricular tachycardia (VT) and ventricular arrhythmia (VA), time to sinus rhythm (SR) and duration of QRS complexes. The QRS complexes were measured by manual positioning of the screen markers. At least four complexes were measured and averaged at each time point.

Statistical analysis

The values of parameters measured are presented as mean±s.d. for the indicated number (n) of experiments. Results were analysed using the software package GraphPad Prism Version 2.0 and GraphPad InStat (GraphPad Software Inc., San Diego, CA). All tests were performed on raw data obtained from the experiments (n = 6). The effect of a single qualitative factor on a single response variable was determined by analysis of variance using the Tukey's post-hoc test. P values < 0.05 were accepted as evidence of a statistically significant difference.

Results and Discussion

Whole-cell patch-clamp method

Calcium-channel activity

In recent years, Ca^{2+} antagonists have found increasing favour in the treatment of cardiovascular disorders, such as cardiac arrhythmias. Their use is largely based on the disruption of voltage-dependent Ca^{2+} channels in both the cardiac muscle and vascular smooth muscle (Dong et al 1993). Some substances that inhibit Ca^{2+} channels may have a lowered negative inotropic effect, possibly as a result of influences on other ion channels (Sensch et al 2000). For this reason, the effect of the isomers on Na^+ and inward rectifying K^+ channels was also determined.

The effects of the isomers on Ca^{2+}-channel activity are summarized in Table 1.

On exposure of the guinea-pig isolated myocyte to 100 μM cyclo(L-Trp-L-Pro), an increased current was observed after a 5-min exposure period at a holding potential of −90 mV (Figure 2). The effect was not completely reversible after a 5-min washout period. The current did not return to control values even if a much longer washout period was allowed. The increase in the current was observed over the whole range of test potentials. The effect seemed to be less pronounced at membrane potentials positive to 5 mV. The peak inward current was observed at 5–15 mV in controls and 0–5 mV after exposure to cyclo(L-Trp-L-Pro). The mean current was increased by 82% at 5 mV. Fitting the current–voltage relationship, $g(E-ENa)/(1+\exp(-(E-Eh)/s))$, revealed that the isomer shifted the reversal potential with 3.8 mV to more positive membrane potentials ($ECa_{control}$ = 32.6 mV; $ECa_{cyclo(L\text{-}Trp\text{-}L\text{-}Pro)}$ = 36.4 mV) and the voltage dependence of activation to more negative membrane potentials ($V1/2_{control}$ = 1.7 mV; $V1/2_{cyclo(L\text{-}Trp\text{-}L\text{-}Pro)}$ = −6.3 mV).

The dependence on the holding potential was determined by activating Ca^{2+} currents from a holding potential of −45 mV. When a holding potential of −45 mV was

Table 1 Effects of the isomers on L-type Ca^{2+}-, Na^{+}- and K^{+}-channel activity in guinea-pig isolated myocytes.

Compound	Ca^{2+}-channel activity[a]		Na^{+}-channel activity[b]	K^{+}-channel activity[c]
	HP = −90 mV 100 μM	HP = −45 mV	100 μM	10 μM
Cyclo(L-Trp-L-Pro)	Agonist (82%)	Antagonist (58%)	Antagonist (37%)	Antagonist (7–10%)
Cyclo(L-Trp-D-Pro)	Agonist (123%)	Antagonist (85%)	Antagonist (52%)	No significant effect
Cyclo(D-Trp-L-Pro)	Antagonist (23%)	Antagonist (36%)	Antagonist (41%)	Antagonist (9–14%)
Cyclo(D-Trp-D-Pro)	Agonist (42%)	Antagonist (29%)	Antagonist (84%)	No significant effect

Percentages in parentheses represent: [a]the increase or decrease in mean current at 5 mV after 5-min exposure to the isomer (e.g. when a holding potential of −90 mV was applied to the cells, the mean current was increased by 82% at 5 mV after 5-min exposure to cyclo(L-Trp-L-Pro) and when a holding potential of −45 mV was applied to the cells, the average peak inward current measured at 5 mV decreased by 58% after exposure to cyclo(L-Trp-L-Pro)); [b]the average decrease in current at a test potential of −30 mV; [c]the inward rectifier K^{+} current between −110 and −140 mV.

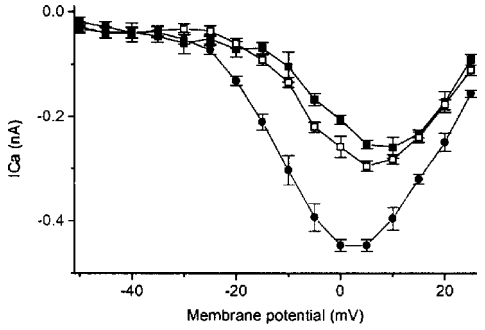

Figure 2 The current–voltage relationship of inward currents in guinea-pig isolated myocytes recorded with the addition of 100 μM cyclo (L-Trp-L-Pro). ■, Control after 10 min (to ensure stable current); ●, 100 μM cyclo(L-Trp-L-Pro) for 5 min; □, washout period of 5 min. Mean currents±s.d. are shown (n = 5).

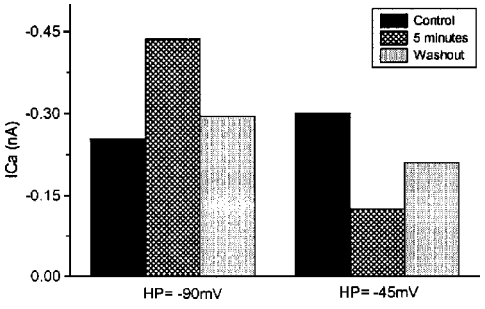

Figure 3 Bars showing the effect of cyclo(L-Trp-L-Pro) at different holding potentials in guinea-pig isolated myocytes. The bars represent mean values (n = 5) for control (−0.25±0.007 nA); cyclo(L-Trp-L-Pro) (−0.44±0.01 nA) and washout (−0.29±0.01 nA) at −90 mV and at −45 mV for control (−0.3±0.04 nA), cyclo(L-Trp-L-Pro) (−0.13±0.02 nA) and washout (−0.21±0.03 nA).

applied to the cells, the peak inward current measured at 5 mV decreased (Figure 3) by 58% after exposure to cyclo(L-Trp-L-Pro).

It is clear from Figure 3 that the effect is dependent on the holding potential with an agonistic effect at −90 mV and an antagonistic effect at −45 mV. Similar observations were made with dihydropyridine agonists like BayK8644, nicardipine and (+)-202-791 (Kamp et al 1989; Carmeliet 1991; Fozzard 1992). Compared with BayK8644, the Ca^{2+} channel seems to be less sensitive to cyclo(L-Trp-L-Pro), as seen from the 2.9-fold increase in the Ca^{2+} current with 1 μM BayK8644 observed by Tiaho et al (1990).

When 100 μM cyclo(L-Trp-D-Pro) was applied to a cell held at −90 mV, an increase in the current was observed after the 5-min exposure period. The effect was not completely reversible although the current decreased upon washout. The peak inward current was observed at 0 mV in the control and after exposure to cyclo(L-Trp-D-Pro). The mean current (n = 5) was increased by 114% at 0 mV and 123% at 5 mV. The reversal potential was slightly affected by the isomer ($ECa_{control}$ = 40.3 mV; $ECa_{cyclo(L\text{-}Trp\text{-}D\text{-}Pro)}$ = 38.9 mV). The voltage dependence of activation was shifted to more negative membrane potentials ($V1/2_{control}$ = −4.9 mV; $V1/2_{cyclo(L\text{-}Trp\text{-}D\text{-}Pro)}$ = −11.4 mV). At a holding potential of −45 mV, the inward current measured at 5 mV decreased by 85%.

On application of 100 μM cyclo(D-Trp-L-Pro) a decrease of the inward current at 5 mV was observed. The mean current was decreased by 23% (n = 5). The reversal potential was shifted to less positive membrane potentials ($ECa_{control}$ = 43.0 mV; $ECa_{cyclo(D\text{-}Trp\text{-}L\text{-}Pro)}$ = 36.5 mV). As expected for the reduction in the current the voltage dependence of activation was shifted to depolarized membrane potentials ($V1/2_{control}$ = −3.2 mV; $V1/2_{cyclo(D\text{-}Trp\text{-}L\text{-}Pro)}$ = 2.0 mV). A decrease of 36% in the current was also noted at a holding potential of −45 mV.

Unlike cyclo(L-Trp-L-Pro) and cyclo(L-Trp-D-Pro), it appears that the effect of cyclo(D-Trp-L-Pro) is independent of the holding potential, since antagonism of the Ca^{2+} current was noted at both holding potentials.

A mean increase (n = 5) of 42% at 5 mV and 38% at 10 mV was observed after exposure to cyclo(D-Trp-D-Pro). The reversal potentials were shifted to more positive membrane potentials ($ECa_{control}$ = 39.8 mV; $ECa_{cyclo(D\text{-}Trp\text{-}D\text{-}Pro)}$

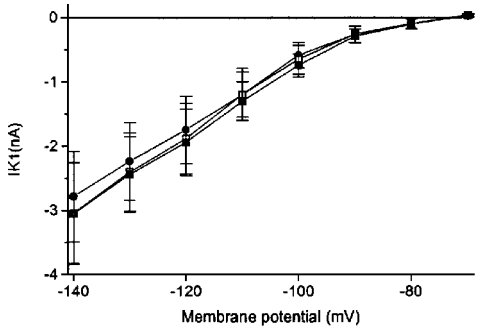

Figure 4 The current–voltage relationship of inward rectifying K^+ currents (n = 4) recorded in guinea-pig isolated myocytes with the addition of 100 μM cyclo(L-Trp-L-Pro). ■, Control currents; ●, 100 μM cyclo(L-Trp-L-Pro) for 5 min; □, washout period of 5 min.

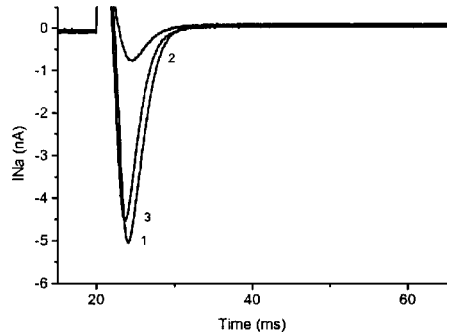

Figure 5 Sodium currents in guinea-pig isolated myocytes recorded from a holding potential of −90 mV to a test potential of −30 mV, after 5 min exposure to cyclo(D-Trp-D-Pro) 10 μM. 1, Control; 2, isomer (10 μM); 3, washout after 5 min.

= 45.1 mV) and the voltage dependence of activation slightly to more negative membrane potentials ($V1/2_{control}$ = 3.2 mV; $V1/2_{cyclo(D\text{-}Trp\text{-}D\text{-}Pro)}$ = 1.13 mV). At a holding potential of −45 mV, the current was decreased by 29% after the 5-min exposure period.

In a previous study, the effect of cyclo(Trp-Pro) on Ca^{2+}-channel activity was determined and it was found that this compound resulted in a 45% blockage of the inward Ca^{2+} current after a 1-min exposure. This was increased to 50% after a 3-min exposure. Furthermore, cyclo(Trp-Trp) showed antagonistic activity towards Ca^{2+}-channel activity, with 45% blockage after a 1-min exposure to 100 μM solution of the dipeptide. This blockage increased by 2% to 47% after a 3-min exposure and was found to be faster acting than cyclo(L-Trp-L-Pro) (Milne et al 1998).

Potassium-channel activity

Figure 4 shows inward rectifying K^+ currents (n = 4) at membrane potentials between −140 and −70 mV. Exposing the cell to 100 μM cyclo(L-Trp-L-Pro) for 5 min caused a small decrease (7–10%) in the current at hyperpolarized potentials. The effect was reversible, with the current returning to control values after the 5-min washout period.

The inward rectifier K^+ current was decreased between −140 and −110 mV (9%, 9% and 14%, respectively) after a 5-min exposure period to cyclo(D-Trp-L-Pro). The effect was completely reversible after the 5-min washout period. No significant effect was noted after exposure to cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro).

The effects of the isomers on K^+-channel activity are summarized in Table 1.

The initial study on the effects of cyclo(Trp-Pro), cyclo (Tyr-Pro), cyclo(Phe-Pro) and cyclo(Trp-Trp) on K^+-channel activity was conducted by Milne et al (1998). They found that cyclo(Trp-Pro) and cyclo(Tyr-Pro) inhibited the delayed-rectifier K^+ channels, with cyclo(Tyr-Pro) exhibiting a greater effect (65% as opposed to 38% after a 2-min exposure period). Cyclo(Trp-Trp) and cyclo(Phe-Pro) had no effect on the current. It was further concluded that these compounds did not affect other K^+ channels, such as the inward rectifier current (Milne et al 1998).

Sodium-channel activity

Figure 5 shows Na^+ currents recorded in 20 mM extracellular Na^+ and 0.2 μM nisoldipine, added to block Ca^{2+} channels. All the isomers reduced the Na^+ current after the 5-min exposure time. The currents returned almost to the control level after the washout period. The Na^+ channel was much more sensitive to the isomers than were the Ca^{2+} and K^+ channels. At 100 μM, the current was completely abolished within 30 s of exposure to the isomers. For this reason, a 10-times lower concentration (10 μM) was used. Cyclo(L-Trp-L-Pro) decreased the current by 37%, cyclo(L-Trp-D-Pro) by 52%, cyclo(D-Trp-L-Pro) by 41% and cyclo(D-Trp-D-Pro) by 84% (Figure 5). The same observation was made in 3 separate cells for each isomer.

The effects of the isomers on Na^+-channel activity are summarized in Table 1.

Heart rate

As a result of the agonistic effect of cyclo(L-Trp-L-Pro), cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro) on Ca^{2+}-channel activity, the effect on the heart rate was investigated, as it was suggested that these agonists might possess positive chronotropic activity (i.e. cause an increase in heart rate). On the other hand, due to the antagonistic effect of the DL isomer on Ca^{2+}-channel activity, it was expected that it would possess negative chronotropic activity (i.e. result in a decreased heart rate). In addition, an increase in the coronary flow is expected with the application of cyclo(D-Trp-L-Pro) (Cook 1998).

There was a slight decrease in heart rate in the control sample, which was to be expected, since experimental conditions were not ideal. Cyclo(L-Trp-L-Pro) and cyclo(D-Trp-D-Pro) did not show any significant difference in heart rate when compared with the control ($P > 0.05$). Cyclo(L-Trp-D-Pro) increased the heart rate in relation to the control ($P < 0.05$). As expected, cyclo(D-Trp-L-Pro) decreased the heart rate ($P < 0.05$) significantly.

Coronary flow

During all experiments, coronary flow rates were determined. Common to all Ca^{2+} antagonists is dilation of the coronary vessels (i.e. an increase in coronary flow) (Bova et al 1997). It was thus expected that cyclo(D-Trp-L-Pro) produced increased rates of coronary flow as a result of its antagonist action against Ca^{2+} channels. Neither cyclo(L-Trp-L-Pro) nor cyclo(D-Trp-D-Pro) produced any significant effects on the coronary flow in relation to the control ($P > 0.05$). Both cyclo(L-Trp-D-Pro) and cyclo(D-Trp-L-Pro) increased coronary flow when compared with the control samples, although these levels were not statistically significant ($P > 0.05$). The increase in coronary flow produced by cyclo(D-Trp-L-Pro) was expected, although a decrease in coronary flow with cyclo(L-Trp-D-Pro) was anticipated. Of particular interest is the fact that cyclo(L-Trp-L-Pro) and cyclo(D-Trp-D-Pro) did not produce any significant effects on cardiac vascular smooth muscle (from coronary flow rate results). This indicates that cyclo(L-Trp-D-Pro) and cyclo(D-Trp-L-Pro) resulted in relaxed vascular smooth muscle, which caused blood vessels in these regions to dilate, resulting in an increased coronary flow (Nayler 1988). Disease states such as angina and myocardial infarction are often accompanied by arrhythmias, which may be aggravated by drugs that cause vasoconstriction. It is thus of particular interest that none of the isomers tested resulted in vasoconstriction (i.e. a decrease in coronary flow rate).

Duration in VT and VA, and time to SR

A major cause of morbidity and mortality is VA, which is associated with myocardial ischaemia. No complete therapeutic solution is available as yet (Barrett et al 2000). In all experiments, the coronary flow decreased to less than 40% during occlusion and returned to normal after reperfusion. The time spent in VT and VA, and the time to SR (Figure 6) were determined by studying the ECGs.

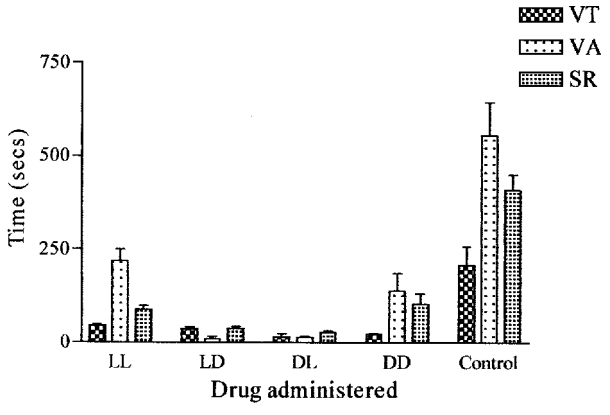

Figure 6 The time to stop VT (ventricular tachycardia), VA (ventricular arrhythmias) and time to return to SR (normal sinus rhythm) in rat isolated heart in the presence of 200 μM LL (cyclo(L-Trp-L-Pro)), LD (cyclo(L-Trp-D-Pro)), DL (cyclo(D-Trp-L-Pro)) and DD (cyclo(D-Trp-D-Pro)).

The time spent in VT (Figure 6) was significantly reduced in the presence of all the isomers in comparison with the control ($P < 0.05$ for all the isomers). Similarly, time spent in VA was also significantly reduced for all the isomers ($P < 0.01$ for cyclo(L-Trp-L-Pro), and $P < 0.001$ for cyclo(L-Trp-D-Pro), cyclo(D-Trp-L-Pro) and cyclo(D-Trp-D-Pro)). In addition, the time taken to return to SR was greatly reduced in comparison with the control group ($P < 0.001$ for all the isomers). These results show that significant reduction in the severity of arrhythmias that result from coronary artery ligation can be achieved with all the isomers of cyclo(Trp-Pro).

QRS intervals

No significant alterations in the duration of the QRS intervals were observed for any of the isomers when compared with the control samples ($P > 0.05$), with the exception of cyclo(D-Trp-D-Pro) ($P < 0.05$). Cyclo(D-Trp-L-Pro) showed a significant decrease in QRS complex duration in comparison with cyclo(D-Trp-D-Pro) ($P < 0.001$), indicating a decreased intraventricular conduction time when the effects of the two isomers were compared.

Conclusions

Arrhythmias are of particular concern, as contractions that are too fast, asynchronous or too slow will reduce cardiac output. Arrhythmias may precipitate further complications in the form of VFs. Any agent capable of reducing the duration of VA and VF are of considerable importance in the sense that the agent is capable of modifying critically impaired conduction. Of the isomers tested, only cyclo(D-Trp-L-Pro) showed potential as a Ca^{2+}-channel antagonist, which is favoured by depolarizing potentials. Cyclo(L-Trp-L-Pro), cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro) all showed Ca^{2+}-channel agonism, which is favoured by hyperpolarizing potentials. Only cyclo(D-Trp-L-Pro) showed independence of membrane potential. No effect on the inward rectifier K^+ current was noted for cyclo(L-Trp-D-Pro) and cyclo(D-Trp-D-Pro), while cyclo(L-Trp-L-Pro) and cyclo(D-Trp-L-Pro) showed antagonistic activity. Furthermore, the isomers affected Na^+-channel activity significantly, with a higher affinity than for the Ca^{2+} channel (cyclo(D-Trp-D-Pro) showed the greatest effect on the Na^+ channel). This reduction in Na^+ influx can result in a reduction in the force of the cardiac contraction, since the Na^+ load of a cell contributes by means of the Na^+/Ca^{2+}-exchanger to the Ca^{2+} load of the cell.

An agent commonly used for drug-resistant arrhythmias, amiodarone, possesses K^+, Na^+, Ca^{2+} and non-specific sympathetic blocking effects, and is thus non-selective in its action. It should therefore be emphasized that the purpose of the ion-channel experiment was to test for selectivity and effects that may be advantageous for use as antiarrhythmic agents.

In certain types of vascular disease (e.g. ischaemia), heart cells tend to be relatively depolarized and may therefore be more susceptible to Ca^{2+} antagonists working at depolarized potentials. Cyclo(L-Trp-L-Pro) and cyclo(D-

Trp-D-Pro) showed no significant effect on the heart rate, while cyclo(L-Trp-D-Pro) showed a positive chronotropic effect, with cyclo(D-Trp-L-Pro) exhibiting negative chronotropic effects. These effects were, however, not statistically significant. Clinical research has shown that vasodilators are capable of relieving the stressed myocardium by reducing the vascular tone, and have thus shown considerable success in the treatment of some types of congestive failure (Hondeghem & Mason 1989). No significant increase in coronary flow was observed for any of the isomers. Cyclo(L-Trp-D-Pro) was expected to decrease the coronary flow, since it showed positive chronotropic effects. One should also bear in mind that vascular smooth muscle is known to have a less negative resting potential than heart muscle. This would effect a relaxed coronary vasculature without significantly depressing cardiac contracture. To clarify this, it is suggested that studies be conducted on guinea-pig isolated hearts, to eliminate any discrepancies as far as species differences are concerned.

Furthermore, it was found that all the isomers were capable of reducing the time spent in both VA and VT, as well as reducing the time taken for the heart to return to SR. These isomers show potential as antiarrhythmic agents and should be investigated further, as coronary flow is not decreased on application, and will thus not place extra strain on the heart.

Subsequent investigation will focus on the mechanism of interaction with the channels, where K_d values, association and dissociation rates, frequency dependence, etc., will be studied.

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