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Antiarrhythmic effects of DPI 201–106

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1 The cardiotonic agent DPI 201–106 (4-[3-(4-diphenylmethyl-1-piperazinyl-2-hydroxypropyl]-1H-indole-2-carbonitrile) which modifies the sarcolemmal Na^+ channel gating system and has electrophysiological properties of class III antiarrhythmics was investigated for local anaesthetic and antiarrhythmic activity.

2 The compound action potential amplitude of cat cervical vagus nerves *in vitro* was decreased by DPI 201–106 in a concentration-dependent manner, the IC_{50} being 1.82×10^{-5} M. This was paralleled by a slowing in conduction velocity and demonstrates local anaesthetic effects.

3 Ventricular fibrillation which occurs in response to coronary artery reperfusion in rats was prevented by intravenous infusions of 0.3 mg $kg^{-1}min^{-1}$ of DPI 201–106.

4 The arrhythmogenic intravenous doses of aconitine in rats were increased following pretreatment with DPI 201–106 in a dose-dependent manner.

5 DPI 201–106 did not protect against ouabain-induced arrhythmias in guinea-pigs.

6 The results demonstrate that DPI 201–106 has local anaesthetic effects and is a potential antiarrhythmic.

Introduction

The cardiotonic DPI 201–106 (4-[3-(4-diphenylmethyl-1-piperazinyl-2-hydroxypropyl]-1H-indole-2-carbonitrile) (DPI) recently underwent clinical trials in the treatment of cardiac failure (Thormann *et al.*, 1985). The compound exerts a spectrum of pharmacological effects which comprise increase in force of contraction, dilatation of coronary vessels, decrease in spontaneous heart rate and prolongation of the action potential duration (APD) (Scholtysik *et al.*, 1985). On the basis of the increase in force of contraction, DPI was developed as a cardiotonic. Cardiotonics can induce or aggravate arrhythmias as is known for Na^+/K^+ ATPase inhibiting digitalis glycosides (Haustein, 1982) and for adenosine 3': 5'-cyclic monophosphate (cyclic AMP) elevating drugs (Opie & Lubbe, 1979; Kanayama *et al.*, 1982; Podzuweit *et al.*, 1985). The mechanism of the positive inotropic effect of DPI is prolongation of the open state of sodium channels (Buggisch *et al.*, 1985) and a sensitization of contractile proteins to Ca^{2+} (Herzig & Quast, 1984) without involvement of cyclic AMP (Scholtysik *et al.*, 1985). Since the inotropic mechanism of DPI differs from that of previously developed cardiotonics it may not suffer from similar side effects. Furthermore antiarrhythmics of class III are characterized by a prolongtion of the APD (Bexton & Camm, 1982) and as this is also a property of DPI it may even act as an antiarrhythmic. For this reason DPI was studied in animal models for antiarrhythmic activity. Furthermore DPI was examined for local anaesthetic effects in neurones since it has affinity for Na^+ channels.

The results show that DPI is a potent local anaesthetic in cat isolated vagus nerves and protects against arrhythmias induced by coronary occlusion/reperfusion and aconitine in rats but was ineffective against those induced by ouabain in guinea-pigs.

Methods

Local anaesthetic test

Adult male or female cats weighing 3–4 kg were anaesthetized with chloralose/urethane (430/43 mg kg^{-1}, i.p.). Both cervical vagus nerves were dissected. The nerves were placed in a Petri dish containing oxygenated Ringer solution of the following composition (mM): $CaCl_2$ 2.25, KCl 5.6, NaCl 154, brought to pH 7.6 with NaOH. Under the microscope (16 ×) the nerves were completely desheathed and mounted horizontally in a compartmentalised Perspex chamber as shown in Figure 1 (modification after

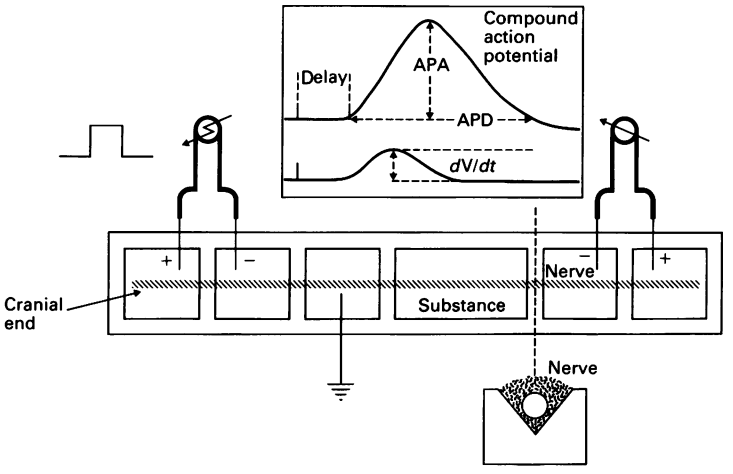

Figure 1 Schematic presentation of the method employed to detect local anaesthetic activity in cat cervical vagus nerves.

Neto, 1978). The compound action potentials were elicited by means of platinum electrodes and a Grass S 88 stimulator. Supramaximal pulses of 0.05 ms duration were delivered every 2 s. The action potentials were displayed on a Tektronix 7623A oscilloscope and photographed. The maximum upstroke velocity was determined by an analog differentiator. Measurements were: action potential amplitude (APA) in ms, action potential duration (APD) in ms, upstroke velocity (dV/dt_{max}) in V s^{-1} and the conduction delay in ms between stimulus artefact and compound action potential as an indicator of conduction velocity.

Coronary artery occlusion- and reperfusion-induced arrhythmias in anaesthetized rats

Male Wistar rats (240–390 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.). The femoral vein was cannulated to allow drug administration, and the trachea for artificial respiration. Systemic blood pressure was monitored from the carotid artery with a Statham P23 1D transducer. A standard lead I ECG was recorded together with systemic blood pressure.

The chest was opened by a left thoracotomy, followed by sectioning of ribs 4 and 5, approximately 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 ml 100 g^{-1} and a rate of 54 beats min^{-1}. After incising the pericardium, the heart was eased out of the chest, gentle pressure being applied to the rib cage. A polyamide suture attached to a cutting needle was placed under the left main coronary artery. The heart was replaced in the chest and the animal left to recover for 15 min. Any animal in which this procedure produced arrhythmias or a sustained fall in blood pressure to <70 mmHg was discarded.

A small plastic button was threaded through the ligature and placed in contact with the heart. The artery could then be occluded by applying tension to the ligature, and reperfusion achieved by releasing the tension. The artery was occluded for 5 min before release (Williams *et al.*, 1985).

Evaluation of reperfusion-induced arrhythmias Within seconds of releasing the occlusion, ventricular tachycardia (VT) occurs and often degenerates into ventricular fibrillation (VF) (Kane *et al.*, 1984). This is not always a terminal event in rats, since spontaneous reversion to sinus rhythm can occur. If VF is not terminal the arrhythmias terminate within a few minutes. The percentage incidence of ventricular ectopic beats (VEB), of VT and of VF, was noted during both the occlusion and reperfusion periods together with the mortality.

Control (solvent) studies were performed alongside each drug study. DPI 201–106 was administered in doses of 0.1 and 0.3 mg $kg^{-1}min^{-1}$ and 3.0 mg kg^{-1} 10 min before temporary occlusion of the coronary artery.

Aconitine-induced arrhythmias in rats

Female rats of the strain OFA and body weight of 210–250 g were anaesthetized with urethane, 1.5 g kg^{-1} i.p. The trachea and the jugular vein were cannulated. Needle electrodes were inserted subcutan-

eously in the limbs and the ECG was recorded on a three-channel Mingograph and displayed on an oscilloscope. Aconitine was infused intravenously at a rate of $5\,\mu g\,kg^{-1}\,min^{-1}$ ($0.1\,ml\,min^{-1}$) which induced arrhythmia in all animals. The arrhythmia induced by aconitine was observed in the ECG recordings. The doses of aconitine which produced extrasystoles, VT and cardiac arrest persisting for at least 5 s were determined.

Groups of 10 animals were pretreated with increasing doses of DPI 5 min before the aconitine infusion was started. The control animals received saline. For each animal, the aconitine dose required to induce arrhythmia was recorded as the endpoint. The endpoint doses in animals pretreated with DPI were compared with the mean + 2 s.d. of the control group. For each dose, an endpoint value greater than mean + 2 s.d. of the corresponding control values was taken as a positive response (animal protected). The percentage response was converted to a probit of response, and a dose-probit curve was constructed from which an ED_{50} value was calculated (Finney, 1971). The ED_{50} value is defined as the dose which protects 50% of the rats against aconitine arrhythmia. The method has been described previously (Scholtysik, 1981).

Ouabain-induced arrhythmias in guinea-pigs

Female albino guinea-pigs weighing 360–460 g were anaesthetized with urethane $1.5\,g\,kg^{-1}$ intraperitoneally. The ECG leads I, II and III were recorded from subcutaneous needle electrodes. Catheters were inserted into a jugular vein for drug administration and a carotid artery for blood pressure measurement. Ouabain was infused intravenously at a rate of $20\,\mu g\,kg^{-1}\,min^{-1}$ until death occurred. Doses of ouabain inducing VT, VF and death were noted. Groups of guinea-pigs were treated intravenously with saline or DPI 5 min before the start of ouabain infusion.

Results

Local anaesthetic activity

In cat vagus nerves DPI decreased APA and *dV/dt* of compound action potentials and increased conduction delay. All effects were concentration-dependent from 3×10^{-6} M to 3×10^{-5} M (Figure 2). APD was not influenced by DPI. A 50% reduction of APA (EC_{50}) was obtained with 1.82×10^{-5} M of DPI. The EC_{50} for amethocaine and lignocaine were 4.66×10^{-5} M and 1.26×10^{-3} M respectively when tested by us in this model. Thus DPI proved to be a comparatively potent local anaesthetic.

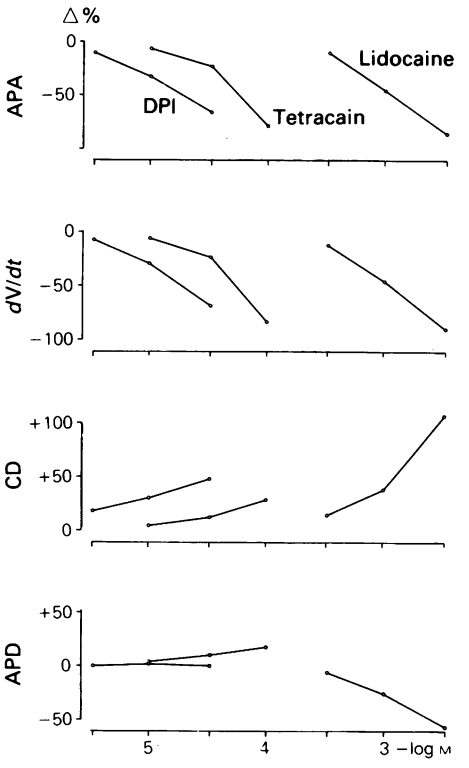

Figure 2 Compound action potentials of cat cervical vagus nerves as affected by DPI 201–106 ($n = 5$), amethocaine ($n = 8$) and lignocaine ($n = 8$). Presented are percentage deflections from pretreatment measurements of action potential amplitude (APA), maximum rate of rise of the action potential (*dV/dt*), conduction delay (CD) and action potential duration (APD).

Prevention of coronary occlusion and reperfusion-induced arrhythmias in rats

In initial experiments DPI was administered in a bolus dose of $3\,mg\,kg^{-1}$ i.v. The incidence of ventricular arrhythmias during the periods of ischaemia and reperfusion is listed in Table 1. The incidence of VEB and VT during the ischaemic period was lower in animals receiving DPI, although the difference was not statistically significant, and there were no deaths. The severity of reperfusion-induced arrhythmias was similar in both groups. Administration of DPI resulted in a marked fall in both arterial blood pressure and heart rate (Figure 3). Changes in the lead I ECG were also observed. The QT interval was prolonged. However, this action of DPI was relatively transient, with a return towards control values after 15 min. Consequently an infusion of DPI was performed in a second experimental series.

Table 1 The percentage incidence of ventricular ectopic beats (VEB), ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality during 5 min of coronary artery occlusion and after reperfusion in anaesthetized rats

		Occlusion				*Reperfusion*			
Treatment	n	VEB	VT	VF	Mortality	VEB	VT	VF	Mortality
Solvent control	10	90	50	10	10	100	89	56	56
DPI 201–106 (3 mg kg^{-1})	10	50	10	0	0	90	70	50	20
Solvent control	11	73	27	9	9	100	100	70	60
DPI 201–106 (0.1 mg kg^{-1} min^{-1})	10	30	20	0	0	80	70	50	20
DPI 201–106 (0.3 mg kg^{-1} min^{-1})	10	0	0	0	0	20***	10***	0**	0**

**$P < 0.01$; ***$P < 0.001$ Fisher's Exact Test

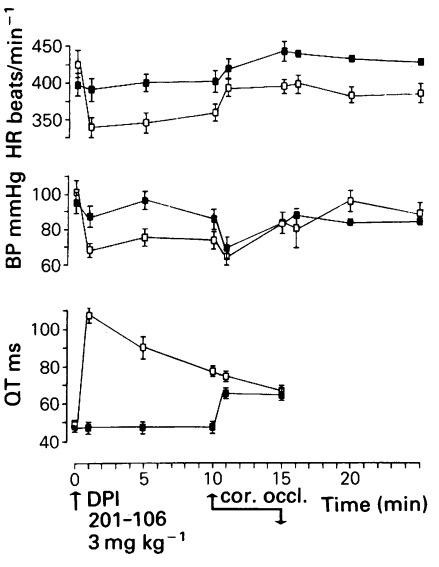

Figure 3 Time course of the effect of DPI 201–106 (□) on heart rate (HR), mean arterial blood pressure (BP) and QT-interval in the ECG (QT) in anaesthetized rats. Control rats (■) received solvent. Mean values of 10 rats per group; s.e.mean shown by vertical line. Arrows denote the time of coronary artery occlusion and reperfusion.

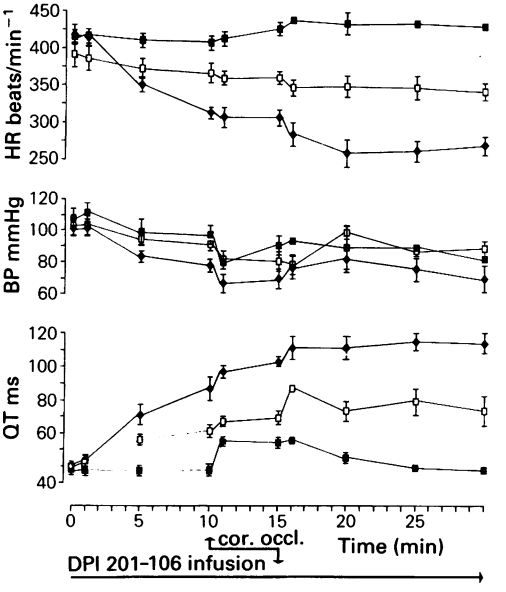

Figure 4 Effects of 30 min i.v. infusions of DPI 201–106 (0.1 (□) or 0.3 (◆) mg kg^{-1}min^{-1}) or solvent (■) on heart rate (HR), mean arterial blood pressure (BP) and QT-interval in the ECG in anaesthetized rats. Mean values of 11 (control) and 10 (each of the DPI-treated group) experiments; s.e.mean shown by vertical lines. Arrows denote the time of coronary artery occlusion and reperfusion.

The incidence of ventricular arrhythmias resulting from occlusion and reperfusion during i.v. infusion is listed in Table 1. The lower infusion rate of 0.1 mg kg^{-1} min^{-1} slightly reduced mortality and the incidence of arrhythmias but the reduction was not statistically significant when compared to controls. No deaths or VF occurred in animals treated with the higher dose of 0.3 mg kg^{-1} min^{-1}. Furthermore the incidence of VT and VEB was also drastically reduced. The infusion of DPI was accompanied by a dose-

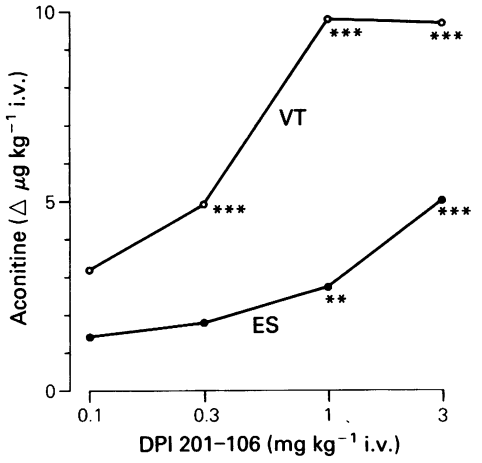

Figure 5 Antiarrhythmic action of DPI 201–106 in anaesthetized rats (n= 5 per dose). Increase in the amount of aconitine required to induce extrasystoles (ES) or ventricular tachycardia (VT). Statistically significant difference from saline-treated controls: $*P<0.05$; $**P<0.01$; $***P<0.001$. Student's t test.

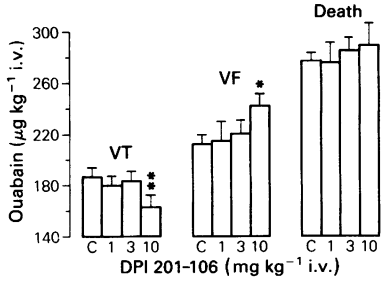

Figure 6 Ouabain toxicity in anaesthetized guinea-pigs. C = saline control, n = 9, DPI 201–106, n = 6 per dose. Amount of ouabain (mean with vertical lines showing s.e.mean) required to induce ventricular tachycardia (VT), ventricular fibrillation (VF) and exitus. Significant difference from control: $*P<0.05$; $**P<0.01$; Student's t test.

dependent reduction in heart rate, arterial blood pressure and a prolongation of the QT interval (Figure 4).

Aconitine antagonism

DPI dose-dependently delayed the onset of arrhythmias in response to aconitine infusion. The calculated (see method) ED_{50} for protection against ventricular extrasystoles and ventricular tachycardia were 0.97 and 0.36 mg kg^{-1} i.v. respectively. The increase in the amount of aconitine required to induced arrhythmias was dose-dependent (Figure 5).

Influence of DPI on ouabain toxicity

Doses of DPI up to 3 mg kg^{-1} i.v. did not influence the toxic effects of ouabain (Figure 6). After the high dose of 10 mg kg^{-1} of DPI the amounts of ouabain required to induce VT and VF were statistically significantly decreased and increased respectively. The lethal doses of ouabain remained unchanged by up to 10 mg kg^{-1} of DPI.

Discussion

The characteristic effect of class III antiarrhythmics is prolongation of the cardiac action potential duration (Vaughan Williams, 1975). The underlying mechanism of this phenomenon has not been defined. The novel cardiotonic agent DPI prolongs the APD (Scholtysik *et al.*, 1985) and was therefore investigated in three different models for antiarrhythmic activity. The basic mechanism of the electrophysiological effect of DPI is prolongation of the open state of Na^+-channels (Buggisch *et al.*, 1985; Kohlhardt *et al.*, 1985). Since it acts at the Na^+-channel, DPI is assumed to have a binding site in or near to the channel. Therefore it was of interest to know whether DPI interacts with neuronal Na^+-channels, which was investigated in a test involving local anaesthetic activity.

In our experiments the cat cervical vagus nerve *in vitro* served for the detection of local anaesthetic effect. This nerve has been found to be sensitive to local anaesthetics for different animal species (Nava Rivera *et al.*, 1967; Ritchie & Ritchie, 1968; Gissen *et al.*, 1968). DPI suppressed the compound action potential and delayed the impulse conduction. DPI was 2.5 times as potent as amethocaine and about 70 times as potent as lignocaine. The duration of the compound action potential was not influenced by DPI but increased by amethocaine and decreased by lignocaine. The reason for the differences is not clear. In the case of the cardiac action potential $\dot{V}_{max}$ was somewhat depressed by DPI (Scholtysik *et al.*, 1985; Buggisch *et al.*, 1985). This is in agreement with the neuronal local anaesthetic activity. Nevertheless DPI has an additional effect on cardiac Na^+ channels, namely to prolong the duration of the open state.

Of particular interest is that DPI, when given intravenously, markedly protects against reperfusion-induced cardiac arrhythmias in anaesthetized rats. Infusion of 0.3 mg kg^{-1} min^{-1} reduced the incidence of VF and VT which followed the release of a 5 min occlusion of the left coronary artery. It was necessary to infuse DPI since its duration of action as reflected

by heart rate decrease and ECG effects in rats is transient lasting for only about 15 min after bolus injection. Other antiarrhythmics found to be effective in this model (Kane *et al.*, 1984) include lignocaine (class I) and melperone (class III). The class III effects of melperone were described by Platou *et al.*

Doses of aconitine required to induce arrhythmias in anaesthetized rats were increased following pretreatment with DPI. With an ED_{50} of 0.97 $mg\,kg^{-1}$ i.v., DPI is as potent as lorcainide (ED_{50} 0.66 $mg\,kg^{-1}$ i.v., Scholtysik, 1981).

Arrhythmias induced by cardiac glycosides are prevented by many of the clinically used antiarrhythmics (Winslow, 1984). However, even at a dose of 10 $mg\,kg^{-1}$ i.v., DPI did not protect guinea-pigs against ouabain-induced arrhythmias. The reason for the ineffectiveness of DPI in this model may lie in its mechanism of action. DPI may load cardiac cells with Na^+ (Buggisch *et al.*, 1985). Na^+-load leads to an increase in Na^+/K^+-ATPase activity. This in turn allows an increased binding of the glycoside to its receptor (Na^+/K^+-ATPase). Consequently toxicity is increased. This has been demonstrated by Na^+-load when induced by increases in the stimulation frequency (Kennedy *et al.*, 1983). Due to the Na^+-channel activation DPI could theoretically increase the toxicity of ouabain. This expected effect may be prevented by its antiarrhythmic potential.

In conclusion, local anaesthetic and antiarrhythmic effects add to the potential therapeutic interest of DPI, a compound with cardiotonic and coronary dilator properties. Further investigations are necessary to classify and elucidate its mechanism of antiarrhythmic action.

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