Inotropic and electrophysiological effects of BDF 9148, a congener of DPI 201-106, in guinea-pig atria and papillary muscles

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1 BDF 9148 is a newly synthesized congener of DPI 201-106 in which the piperazidinyl moiety has been replaced by an azetidine-3-oxy-moiety. The inotropic effect of both drugs was studied as well as their influence on the action potential, by use of standard microelectrode techniques.

2 BDF 9148 increased the contractile force of guinea-pig atria and papillary muscles. The EC_{50} was $1.32 \times 10^{-7} \text{ mol l}^{-1}$ and $0.7 \times 10^{-6} \text{ mol l}^{-1}$ respectively. DPI 201-106 was effective in a similar concentration-range, the EC_{50} being $2.6 \times 10^{-7} \text{ mol l}^{-1}$ for atria and $2.8 \times 10^{-7} \text{ mol l}^{-1}$ for papillary muscles.

3 Both drugs caused a concentration-dependent prolongation of the relaxation time of the isometric contraction curve. With $10^{-6} \text{ mol l}^{-1}$ BDF 9148, the mean increase was $39.1 \pm 4.4 \text{ ms}$ in atria and $39.4 \pm 7.5 \text{ ms}$ in papillary muscles while $10^{-6} \text{ mol l}^{-1}$ DPI 201-106 caused increases of $56.7 \pm 2.5 \text{ ms}$ and $79.3 \pm 11.7 \text{ ms}$, respectively. The effect of BDF 9148 was not prevented by propranolol, but was reversed by $3 \times 10^{-6} \text{ mol l}^{-1}$ tetrodotoxin. Pretreatment of atria with $3 \times 10^{-8} \text{ mol l}^{-1}$ BDF shifted the concentration-response curve of ouabain to the left and reduced the EC_{50} of the glycoside from $3.21 \times 10^{-7} \text{ mol l}^{-1}$ to $2 \times 10^{-7} \text{ mol l}^{-1}$.

4 BDF 9148 and DPI 201-106 concentration-dependently increased the action potential duration of papillary muscles and their functional refractory period. The drugs did not modify the resting potential, the action potential amplitude or the maximum depolarization velocity.

5 All effects of BDF 9148 persisted after washout. The lipophilic drug accumulated in the tissues and the tissue drug concentration was little reduced by washout of BDF 9148 from the organ bath.

6 In contrast to DPI 201-106, which had a prominent negative chronotropic effect in right atria, BDF 9148 caused only a slight reduction of the beating frequency with the largest ($3 \times 10^{-5} \text{ mol l}^{-1}$) concentration.

7 The results are consistent with BDF 9148 being a sodium channel activator with much weaker influence on the beating frequency than the parent compound DPI 201-106.

Keywords: BDF 9148; DPI 201-106; inotropic effect; action potential; atrium; papillary muscle

Introduction

Cardiac glycosides are effective in congestive heart failure, but their safety margin is small and toxic side-effects are not uncommon. Inhibitors of phosphodiesterase III have been tried as an alternative therapeutic principle for this disease. However, these drugs are potentially arrhythmogenic because they increase the adenosine 3':5'-cyclic monophosphate (cyclic AMP) level of the myocardial cells. Furthermore, in the failing heart the number of β -adrenoceptors is reduced (Bristow *et al.*, 1982) and the stimulation of adenylate cyclase is impaired by an increased concentration of G_i -proteins (Feldman *et al.*, 1987). Therefore, the effectiveness of phosphodiesterase inhibitors is reduced in end-stage heart failure (Feldman *et al.*, 1987; Böhm *et al.*, 1988), and there is still a need for new positive inotropic drugs which are clinically effective and safe in congestive heart failure.

An increased intracellular sodium concentration inhibits the diastolic calcium extrusion via the sodium-calcium exchange mechanism. Therefore, drugs which increase the sodium influx may increase the contractile force. The positive inotropic effect of naturally occurring sodium channel activators cannot be exploited therapeutically, because these drugs cause a dramatic prolongation of the action potential duration, can trigger afterdepolarizations and thus induce arrhythmias (Honerjäger, 1982). However, the piperaziny-indole DPI 201-106 has been used successfully in some recent clinical trials (Kostis *et al.*, 1987; Butrous *et al.*, 1988; Hogan *et al.*, 1988). BDF 9148, systematic name 4-[3-(1-diphenylmethyl-

azetidine-3-oxy)-2-hydroxy-propoxy]-1-H-indole-2-carbonitrile, is a recently synthesized derivative, where the piperaziny moiety of DPI 201-106 has been replaced by an azetidine-3-oxy-moiety (Figure 1). Several preliminary communications (Armah *et al.*, 1990a,b; Brasch, 1990; Brasch & Iven, 1990) have shown that this new drug increases the contractile force of heart muscle preparations *in vitro*. In the

Figure 1 Chemical structure of BDF 9148 and DPI 201-106.

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present paper we compare the effects of BDF 9148 and DPI 201-106 in guinea-pig isolated atria and papillary muscles.

Methods

Experimental procedure

Male guinea pigs (300–500 g) were killed by a blow on the head. The hearts were rapidly removed and right or left atria or papillary muscles from the right ventricle were dissected in cold, oxygenated Krebs-Henseleit solution. The muscles were then mounted in 20 ml organ baths filled with Krebs-Henseleit solution of the following composition (mmol l^{-1}): NaCl 117.6, KCl 5.8, CaCl_2 1.8, MgSO_4 1.2, NaH_2PO_4 1.2, NaHCO_3 25 and dextrose 5.5. They were kept at $32 \pm 0.2^\circ\text{C}$ and constantly gassed with 95% O_2 and 5% CO_2 . The low temperature was chosen because at 37°C the oxygen demand is greater and many preparations show a marked decline of the contractile force during a control period of some hours. This decline is negligible at 32°C which, therefore, is a commonly used temperature for *in vitro* experiments with heart muscle. The resting tension was adjusted to 10 mN.

Right atria were allowed to beat spontaneously. Left atria and papillary muscles were stimulated at a rate of 1 Hz via platinum electrodes with square pulses of 1 ms duration and 1.5 times threshold voltage. The force of contraction was registered isometrically by a K30 force-displacement transducer (Hugo-Sachs, Hugstetten, F.R.G.) and the isometric contraction curve was displayed on a Hellige Helcoscriptor (Hellige, Freiburg, F.R.G.). The time to peak force (t_1) and the relaxation time (t_2) were measured at 10% of the maximum of the isometric contraction curve. Action potentials (AP) from papillary muscles were recorded with standard glass micro-electrodes which were filled with 3 M KCl and had a tip resistance of 5–10 M Ω . The maximum depolarization velocity $\dot{V}_{\text{max}}$ was obtained by passive signal differentiation. The AP and $\dot{V}_{\text{max}}$ were displayed on a Tektronix 502 A dual-beam oscilloscope (Tektronix Inc., Beaverton, OR, U.S.A.) and photographed with a Grass C4 Camera (Grass Medical Instruments, Quincy, MA, U.S.A.) for subsequent analysis. To determine the functional refractory period (FRP), an extra stimulus was inserted with a variable time interval after every sixth regular stimulus. This extra stimulus was delivered automatically by the two channel stimulator which also produced the continuous train of driving pulses.

Experiments were started after an equilibration period of 60–90 min. Cumulative concentration-response curves for BDF 9148, DPI 201-106 or ouabain were obtained by increasing the drug concentration stepwise in 20 min or 40 min intervals. For the evaluation of action potential changes, only those experiments were included in the study in which micro-electrode recordings could be obtained from the same cell during the whole time course of the experiment.

Assay of BDF 9148

The concentration of BDF 9148 in left atria was measured by high performance liquid chromatography (h.p.l.c.). The atria were removed from the organ bath immediately after the end of the experiment, blotted between filter paper, weighed and stored at -20°C . For analysis, the atria were homogenized in 1 ml isopropanol with an Ultraturrax. Four ml chloroform and 200 μl saturated bicarbonate buffer, pH 9.8, were then added and the mixture shaken for 10 min and centrifuged. Three ml of the chloroform phase was evaporated to dryness under a stream of air. The residue was redissolved in 100 μl of the mobile phase for chromatography which consisted of (v/v) methanol 13%, acetonitrile 30%, distilled water 53.5%, acetic acid 1% and tetrahydrofuran 2.5%; 20 μl was injected onto a RP Select B column (Merck, Darmstadt, F.R.G.). The mobile phase was pumped through the column at a rate of 1 ml min $^{-1}$. BDF 9148 was detected and quantified by mea-

suring the absorbance at 280 nm with a Shimadzu SP-4 UV detector. Calibration curves were obtained by adding 100–1000 ng BDF 9148 to untreated atria.

Drugs

Racemic BDF 9148 and DPI 201-106 were kindly provided by Beiersdorf AG (Hamburg, F.R.G.). The other drugs used were ouabain (Sigma, Deisenhofen, F.R.G.), tetrodotoxin (Sankyo, Tokyo, Japan) and propranolol hydrochloride (Rhein-Pharma, Heidelberg, F.R.G.). The reagents used for h.p.l.c. were of analytical grade and were obtained from Merck (Darmstadt, F.R.G.). BDF 9148 and DPI 201-106 had to be dissolved in dimethyl sulphoxide (DMSO). Microlitre amounts of the stock solutions were added to the organ bath and at the end of a concentration-response curve of either drug, the concentration of DMSO in the bath never exceeded 0.75%. All other drugs were dissolved in double distilled water. Stock solutions of all drugs were prepared daily.

Statistics

Arithmetic means were calculated for the pre-drug values of all parameters and for the drug-induced changes. Student's *t* test for paired data was then used to assess the statistical significance of the observed mean changes. $P \leq 0.05$ for the two-tailed test was fixed as the criterion for acceptance of statistical significance throughout. For the construction of concentration-response curves, the positive inotropic effect of each concentration of BDF 9148, DPI 201-106 or ouabain was expressed as a percentage of the corresponding maximum effect. EC_{50} values were then calculated from the log concentration-effect curves after linearization by probit transformation and estimation of the regression function with the least squares method. Regression lines were compared as described by Documenta Geigy (1968).

Results

Chronotropic effect

At the beginning of the experiments, the beating frequency of right atria ranged between 160 and 170 beats per minute (b.p.m.). Increasing the concentration of BDF 9148 stepwise in 20 min intervals up to $3 \times 10^{-6} \text{ mol l}^{-1}$ caused no significant change of the contraction rate. Only with the final concentration of $10^{-5} \text{ mol l}^{-1}$ a slight reduction to 144 ± 5 b.p.m. was observed (Figure 2). DPI 201-106, in concentrations between $10^{-7} \text{ mol l}^{-1}$ and $10^{-5} \text{ mol l}^{-1}$, caused a concentration-dependent decrease of the beating frequency from 161 ± 5 b.p.m. to 86 ± 7 b.p.m. (Figure 2). The solvent DMSO, which was added cumulatively in 6 control experiments up to a final concentration of 0.75%, did not change the contraction rate significantly (data not shown).

Inotropic effect

Cumulative concentration-response curves (dosing interval 40 min) for the positive inotropic effect of BDF 9148 and DPI 201-106 are shown in Figure 3. In left atria, BDF 9148 increased the force of contraction from 4.3 ± 1.0 mN to 11.5 ± 1.8 mN and DPI 201-106 caused an increase from 2.5 ± 0.5 mN to 10.9 ± 0.7 mN. We cannot explain why the predrug contractile force was so much smaller in the DPI 201-106-treated muscles, but we are confident that a technical mistake in the experiments can be excluded. Due to this discrepancy, the % force increase obtained with BDF 9148 (+167%) was smaller than the % increase produced by DPI 201-106 (+336%) though the maximum developed force was nearly identical with both drugs. Also, the EC_{50} values of the two compounds were not significantly different. For BDF 9148 an EC_{50} of $1.32 \times 10^{-7} \text{ mol l}^{-1}$ was calculated (95%

Figure 2 Influence of BDF 9148 (●) and DPI 201-106 (■) on the beating frequency of right atria. Drugs were added cumulatively in 20 min intervals. Abscissa scale: drug concentrations (mol l^{-1}). Ordinate scale: beating frequency (b.p.m.). C = pre-drug control value. Means and their standard errors (vertical bars) ($n = 10$ for BDF, $n = 6$ for DPI) are shown. * The beating frequency is significantly different from the corresponding predrug control ($P \leq 0.05$; t test for paired data).

confidence interval $0.46\text{--}3.78 \times 10^{-7} \text{ mol l}^{-1}$) and the corresponding value for DPI 201-106 was $2.6 \times 10^{-7} \text{ mol l}^{-1}$ ($2.0\text{--}3.4 \times 10^{-7} \text{ mol l}^{-1}$).

In papillary muscles, BDF 9148 increased the contractile force from $2.0 \pm 0.6 \text{ mN}$ to $4.4 \pm 0.8 \text{ mN}$ (+120%), and DPI 201-106 produced an increase from $2.6 \pm 0.4 \text{ mN}$ to $5.8 \pm 0.8 \text{ mN}$ (+123%). The EC_{50} for BDF 9148, however, ($7 \times 10^{-7} \text{ mol l}^{-1}$; 95% confidence interval $5.6\text{--}8.8 \times 10^{-7} \text{ mol l}^{-1}$) was significantly greater than for DPI 201-106 ($2.8 \times 10^{-7} \text{ mol l}^{-1}$; 95% confidence interval $2.2\text{--}3.5 \times 10^{-7} \text{ mol l}^{-1}$).

Both drugs also changed the shape of the isometric contraction curve. Typical examples are shown in Figure 4. The main effect was a concentration-dependent prolongation of the relaxation time of atria and papillary muscles (Table 1). In both cases, the maximum increase caused by DPI 201-106 was greater than the maximum effect of BDF 9148. The time to peak force of atria was also prolonged by both drugs while the time to peak force of papillary muscles was not affected by BDF 9148 and slightly shortened by DPI 201-106.

Interaction of BDF 9148 with other drugs

After addition of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 to the organ bath, the force of contraction of left atria increased from $4.3 \pm 0.9 \text{ mN}$ to $10.0 \pm 1.2 \text{ mN}$ within 20 min and remained nearly constant thereafter (Figure 5). A similar time course was observed for the prolongation of the relaxation time from $85 \pm 6 \text{ ms}$ to $140 \pm 7 \text{ ms}$. After 1 h of drug exposure, the effects of BDF 9148 could not be reversed or reduced by a 30 min washout period (Figure 5).

In 6 atria, the effect of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 was tested in the presence of $4 \times 10^{-6} \text{ mol l}^{-1}$ propranolol. The β -blocker was added 30 min before BDF 9148 and did not attenuate the positive inotropic effect of the latter. In these experiments the contractile force increased from $2.4 \pm 0.3 \text{ mN}$ to $6.9 \pm 1.0 \text{ mN}$. The relaxation time, however, was prolonged much less (from $80 \pm 3 \text{ ms}$ to $95 \pm 4 \text{ ms}$) than with BDF 9148 alone.

Tetrodotoxin, $3 \times 10^{-6} \text{ mol l}^{-1}$, completely reversed the positive inotropic effect of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 in atria and also the associated increase of the relaxation time (Figure

Figure 3 Cumulative concentration-response curves for the positive inotropic effect of BDF 9148 (a) and DPI 201-106 (b) in left atria (filled symbols) and papillary muscles (open symbols). Means and their standard errors ($n = 6\text{--}8$) are shown. Abscissae: drug concentration (mol l^{-1}). Ordinates: force increase as percentage of the corresponding maximum increase.

Figure 4 Typical examples of the influence of BDF 9148 and DPI 201-106 on the shape of the isometric contraction curve of left atria (a) and papillary muscles (b). Each panel shows the pre-drug control curve and the effect of either BDF 9148 ($10^{-6} \text{ mol l}^{-1}$ in the atrium and $10^{-5} \text{ mol l}^{-1}$ in the papillary muscle) or DPI 201-106 ($10^{-6} \text{ mol l}^{-1}$ in both cases).

Table 1 Influence of BDF 9148 and DPI 201-106 on the time to peak force (t_1) and the relaxation time (t_2) of left atria and papillary muscles

Drug	Left atrium		Papillary muscle	
	t_1 (ms)	t_2 (ms)	t_1 (ms)	t_2 (ms)
Control	48 ± 1.1	189 ± 2.7	111 ± 8.9	164 ± 13.5
BDF 9148 (mol l ⁻¹)				
3 × 10 ⁻⁸	+0.8 ± 1.5	+7.5 ± 3.8	NT	NT
10 ⁻⁷	+3.3 ± 2.1	+15.8 ± 5.8*	NT	NT
3 × 10 ⁻⁷	+6.7 ± 1.1*	+30.8 ± 6.2*	-0.6 ± 6.6	+20.6 ± 5.0*
10 ⁻⁶	+10.8 ± 1.5*	+39.1 ± 4.4*	+1.9 ± 5.8	+39.4 ± 7.5*
3 × 10 ⁻⁶	+9.1 ± 2.0*	+40.0 ± 2.9	+6.9 ± 4.8	+51.9 ± 11.2*
10 ⁻⁵	NT	NT	+3.1 ± 6.0	+48.1 ± 7.1*
Control	49 ± 0.8	87 ± 3.3	140 ± 6.8	171 ± 9.1
DPI 201-106 (mol l ⁻¹)				
10 ⁻⁷	+3.3 ± 3.1	+12.5 ± 5.7	+2.1 ± 3.2	+3.6 ± 9.7
2.15 × 10 ⁻⁷	+1.6 ± 2.1	+15.8 ± 4.4*	-4.3 ± 4.8	+21.4 ± 11.2
4.65 × 10 ⁻⁷	+3.3 ± 2.1	+32.5 ± 5.4*	-4.3 ± 5.2	+45.0 ± 7.6*
10 ⁻⁶	+9.2 ± 3.9	+56.7 ± 2.5*	-10.7 ± 4.9	+79.3 ± 11.7*
2.15 × 10 ⁻⁶	+14.2 ± 1.5*	+58.3 ± 6.0*	-12.1 ± 3.6*	+98.6 ± 12.2*
4.65 × 10 ⁻⁶	+13.3 ± 1.7*	+51.7 ± 6.4*	-17.8 ± 3.2*	+114.3 ± 12.9*

The data were obtained from the cumulative concentration-response curves. Pre-drug values (Control) and their mean changes 40 min after addition of each drug concentration are given ($\bar{x} \pm \text{s.e. mean}$; $n = 6-8$). Asterisks indicate changes that are statistically significant ($P \leq 0.05$; t test for paired data). NT = not tested.

6). In unpretreated muscles (3 preliminary experiments) the concentration of tetrodotoxin used caused a modest (about 20%) reduction of the contractile force and did not change the shape of the isometric contraction curve. The effects of BDF 9148 were reversed within minutes after addition of tetrodotoxin.

However, when both drugs were removed from the organ bath by washout, the force of contraction and the relaxation time started to increase again and reached final values that were similar to the maximum effects observed in the presence of BDF 9148 (Figure 6).

A small concentration of BDF 9148, $3 \times 10^{-8} \text{ mol l}^{-1}$, which by itself caused only a 32% increase of the contractile force, shifted the concentration-response curve for the positive inotropic effect of ouabain in atria to the left (Figure 7). In unpretreated muscles, ouabain increased the force of contraction from $2.7 \pm 0.2 \text{ mN}$ to $12.4 \pm 1.3 \text{ mN}$ and the EC_{50} was $3.21 \times 10^{-7} \text{ mol l}^{-1}$ (95% confidence interval $2.86-3.61 \times 10^{-7} \text{ mol l}^{-1}$). After preincubation with BDF 9148 for 1 h, the maximum effect of ouabain was not affected, i.e. the force increased from $4.71 \pm 0.9 \text{ mN}$ to $12.9 \pm 1.7 \text{ mN}$, but the

EC_{50} was significantly reduced to $2 \times 10^{-7} \text{ mol l}^{-1}$ (95% confidence interval $1.71-2.34 \times 10^{-7} \text{ mol l}^{-1}$).

Tissue concentration of BDF 9148

Two min after the addition of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 to the organ bath, the mean concentration in atria was already $15.3 \pm 3.58 \times 10^{-6} \text{ mol kg}^{-1}$, indicating a 15.3 fold accumulation of the drug in the tissue (Table 2). A prolongation of the incubation time up to 30 min did not increase the tissue concentration of BDF 9148 further. Frequent renewal of the bathing fluid during a 30 min washout period did not reduce the amount of BDF 9148 in the atria significantly; even after 5 h of washout, considerable tissue concentrations of the drug were found.

Figure 5 Influence of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 on the force of contraction (Fc), time to peak force (t_1) and relaxation time (t_2) of left atria. The symbols indicate means and standard errors are shown by vertical bars ($n = 6$). Abscissa scale: time after drug addition (min). W = effect after 30 min washout.

Figure 6 Influence of $10^{-6} \text{ mol l}^{-1}$ BDF 9148 on the force of contraction (Fc), time to peak force (t_1) and relaxation time (t_2) of left atria as it is antagonized by $3 \times 10^{-6} \text{ mol l}^{-1}$ tetrodotoxin (TTX). The symbols indicate means and standard errors are shown by vertical bars ($n = 6$). Abscissae: time after addition of BDF 9148 (min). The drugs were present in the organ bath as indicated by the horizontal bars below the abscissa scale. W = effect after washout (30 min) of both drugs.

Table 2 Concentration of BDF 9148 ($\mu\text{mol kg}^{-1}$) in left atria after incubation with the drug for 2–30 min and subsequent washout

Incubation time	Drug concentration ($\mu\text{mol kg}^{-1}$)		
	Incubation only	Incubation + 30 min washout	Incubation + 5 h washout
2 min	15.30 $\pm$ 3.58	9.36 $\pm$ 0.64	NT
5 min	7.69 $\pm$ 1.70	10.54 $\pm$ 3.25	6.25 $\pm$ 1.57
30 min	18.80 $\pm$ 1.63	19.29 $\pm$ 1.24	NT

Means and their standard errors ($n = 6$) are given. NT = not tested.

Figure 7 Cumulative concentration-response curves for the inotropic effect of ouabain in left atria: (○) ouabain alone; (●) ouabain after pretreatment with $3 \times 10^{-8} \text{ mol l}^{-1}$ BDF 9148. The symbols indicate means and standard errors are shown by vertical bars ($n = 6$). Abscissa scale: concentration of ouabain (mol l^{-1}). Ordinate scale: inotropic effect as a percentage of the corresponding maximum effect.

Effects on action potentials

BDF 9148 and DPI 201-106 increased the duration of action potentials from papillary muscles. Typical examples for this effect are shown in Figure 8. Table 3 lists the detailed results. The concentration of BDF 9148 was increased cumulatively from $3 \times 10^{-7} \text{ mol l}^{-1}$ to $10^{-5} \text{ mol l}^{-1}$ in 20 min intervals and the last concentration stayed in contact with the muscle for 1 h. There were no changes of the resting potential, the amplitude or $\dot{V}_{\text{max}}$ of the action potential. The action potential duration was increased concentration-dependently at all stages of repolarization and the functional refractory period was increased to a similar extent. The effect of BDF 9148 could not be reversed by washout. With DPI 201-106, two successive concentrations were tested which were allowed to act on the muscle for 1 h each. The action potential duration and the functional refractory period were prolonged to the same extent as with equimolar concentrations of BDF 9148. The solvent DMSO, which was added cumulatively in 6 control experiments up to a final concentration of 0.75%, caused no significant change of the action potential parameters (data not shown).

In an additional series of experiments, $10^{-5} \text{ mol l}^{-1}$ BDF 9148 was added to papillary muscles and the effect recorded after 30 min. In this case the prolongation of the action potential duration was much smaller than in the experiments described above ($+16 \pm 2.6 \text{ ms}$ versus $+54 \pm 6.1 \text{ ms}$, see Table 3). In the single concentration experiments, the total duration of the BDF 9148 exposure was shorter than in the cumulative concentration-response experiments (30 min versus at least 80 min), but admittedly this is not a really satisfying explanation for the variability of the drug effect. When

Table 3 Influence of BDF 9148, DPI 201-106 and tetrodotoxin (TTX) on action potential parameters of papillary muscles. Drugs were added cumulatively

		MRP (mV)	APA (mV)	$\dot{V}_{\text{max}}$ (Vs^{-1})	APD ₂₀ (ms)	APD ₈₀ (ms)	FRP (ms)
Control		82 $\pm$ 1.9	111 $\pm$ 0.9	152 $\pm$ 5	131 $\pm$ 5	220 $\pm$ 8	229 $\pm$ 9.0
BDF 9148 (mol l^{-1})							
3×10^{-7}	$\Delta 20 \text{ min}$	-0.3 $\pm$ 0.3	-0.8 $\pm$ 0.5	+1.2 $\pm$ 2.7	+2.0 $\pm$ 2.5	+6.5 $\pm$ 2.3*	+5.0 $\pm$ 2.2
10^{-6}	$\Delta 20 \text{ min}$	+0.7 $\pm$ 0.5	-0.5 $\pm$ 0.8	+4.7 $\pm$ 1.3*	+12.2 $\pm$ 3.5*	+15.5 $\pm$ 4.7*	+21 $\pm$ 5.5*
3×10^{-6}	$\Delta 20 \text{ min}$	+0.5 $\pm$ 0.3	+0.3 $\pm$ 0.7	-0.2 $\pm$ 8.9	+24.3 $\pm$ 5.6*	+33.3 $\pm$ 5.8*	+36 $\pm$ 7.5*
10^{-5}	$\Delta 20 \text{ min}$	+0.8 $\pm$ 0.5	+0.5 $\pm$ 1.4	+2.5 $\pm$ 5.3	+40.2 $\pm$ 6.8*	+54.0 $\pm$ 6.1*	+55 $\pm$ 8.7*
10^{-5}	$\Delta 60 \text{ min}$	+1.0 $\pm$ 0.5	+0.5 $\pm$ 0.9	-0.3 $\pm$ 7.4	+40.2 $\pm$ 6.3*	+58.5 $\pm$ 5.2*	+63 $\pm$ 7.6*
Washout	$\Delta 30 \text{ min}$	-0.3 $\pm$ 0.4	-1.8 $\pm$ 1.3	+4.6 $\pm$ 5.7	+30.5 $\pm$ 5.3*	+54.0 $\pm$ 6.6*	+60 $\pm$ 7.1*
Control		79 $\pm$ 0.6	115 $\pm$ 1.0	149 $\pm$ 7.3	141 $\pm$ 7	214 $\pm$ 12	228 $\pm$ 13.8
DPI 201-106							
3×10^{-6}	$\Delta 20 \text{ min}$	+0.6 $\pm$ 1.2	+0.3 $\pm$ 0.5	-2.1 $\pm$ 4.7	+14.7 $\pm$ 3.9*	+16.3 $\pm$ 4.1*	+15.7 $\pm$ 3.3*
3×10^{-6}	$\Delta 60 \text{ min}$	+0.3 $\pm$ 1.6	0.0 $\pm$ 0.4	+9.7 $\pm$ 6.2	+22.1 $\pm$ 6.2*	+31.0 $\pm$ 6.8*	+33.6 $\pm$ 8.1*
10^{-5}	$\Delta 20 \text{ min}$	+0.4 $\pm$ 1.6	-0.8 $\pm$ 0.8	+0.6 $\pm$ 6.6	+20.8 $\pm$ 7.7*	+46.0 $\pm$ 12.4*	+58.7 $\pm$ 12.0*
10^{-5}	$\Delta 60 \text{ min}$	+1.3 $\pm$ 1.2	-0.6 $\pm$ 0.6	-1.0 $\pm$ 4.6	+30.0 $\pm$ 6.5*	+61.0 $\pm$ 12.4*	+63.6 $\pm$ 13.5*
Control		79 $\pm$ 2.1	113 $\pm$ 1.5	147 $\pm$ 6.2	146 $\pm$ 5.8	245 $\pm$ 12.7	258 $\pm$ 16.6
BDF 10^{-5}	$\Delta 30 \text{ min}$	+0.8 $\pm$ 0.9	+1.0 $\pm$ 0.9	+0.3 $\pm$ 1.3	14.7 $\pm$ 3.7*	+16.0 $\pm$ 2.6*	+25.0 $\pm$ 1.3*
+ TTX 3×10^{-6}	$\Delta 10 \text{ min}$	+1.3 $\pm$ 0.7	+0.7 $\pm$ 1.6	-32.8 $\pm$ 5.0*	-6.7 $\pm$ 3.1	-24.5 $\pm$ 4.4*	0.0 $\pm$ 8.2
Washout	$\Delta 30 \text{ min}$	-0.3 $\pm$ 1.0	0.0 $\pm$ 2.5	-7.0 $\pm$ 6.1	+20.5 $\pm$ 6.4	+27.5 $\pm$ 6.2*	+37.5 $\pm$ 7.4*

The pre-drug values (Control) and their mean changes (Δ) 10, 20, 30 or 60 min after addition of each drug concentration are shown ($\bar{x} \pm \text{s.e. mean}$; $n = 6$). Asterisks indicate changes that are statistically significant ($P \leq 0.05$; t test for paired data). Abbreviations: MRP = membrane resting potential; APA = action potential amplitude; $\dot{V}_{\text{max}}$ = maximum depolarization velocity; APD₂₀, APD₈₀ = action potential duration at 20% and 80% of full repolarization; FRP = functional refractory period.

Figure 8 Typical examples for the influence of BDF 9148 and DPI 201-106 on the action potential of papillary muscles (cumulative drug dosing). (a) Control action potential AP (1) and effect of $3 \times 10^{-7} \text{ mol l}^{-1}$ (2), $10^{-6} \text{ mol l}^{-1}$ (3), $3 \times 10^{-6} \text{ mol l}^{-1}$ (4) and $10^{-5} \text{ mol l}^{-1}$ (5) BDF 9148 after 20 min exposure to each drug concentration. Although there was a small increase of the action potential amplitude with $10^{-5} \text{ mol l}^{-1}$ in this experiment, the mean effect of BDF 9148 on this parameter was not statistically significant (see Table 3). (b) Control AP (1) and effect of $3 \times 10^{-6} \text{ mol l}^{-1}$ (2) and $10^{-5} \text{ mol l}^{-1}$ (3) DPI 201-106 after 60 min exposure to each drug concentration. The corresponding $\dot{V}_{\text{max}}$ values are given in the insets.

$3 \times 10^{-6} \text{ mol l}^{-1}$ tetrodotoxin was added 30 min after $10^{-5} \text{ mol l}^{-1}$ BDF 9148, the influence of the latter was promptly reversed (Table 3). Tetrodotoxin also caused a significant decrease of $\dot{V}_{\text{max}}$. After washout of both drugs, $\dot{V}_{\text{max}}$ was restored and action potential duration and functional refractory period increased again to values slightly above the maximum that was observed in the presence of BDF 9148 beforehand.

Discussion

The positive inotropic effect of the new drug BDF 9148 is not very different from the effect of the parent compound DPI 201-106. Both drugs increased the contractile force of papillary muscles to nearly the same extent. That in atria BDF 9148 produced a much smaller percentage increase of the force than DPI 201-106 should not be overemphasized because the corresponding predrug values were quite different. The maximum force obtained with either compound was nearly identical and the two EC_{50} values were not significantly different in this tissue. But while for DPI 201-106 the EC_{50} was the same in atria and papillary muscles, the sensitivity of the two tissues towards BDF 9148 was different, the EC_{50} being about five fold greater in papillary muscles than in atria. The explanation for this finding is not known.

The inotropic effect of BDF 9148 was not prevented by the β -blocker propranolol. Thus, it cannot be due to a direct stimulation of β -adrenoceptors or to a release of endogenous catecholamines. The associated prolongation of the relaxation time makes it unlikely that the positive inotropic effect is

mediated by an increase of the intracellular cyclic AMP level. A slowing of the relaxation process was also seen with DPI 201-106 and, in general, is a typical finding with drugs which prolong the open state of the sodium channel (Honerjäger, 1982). As the effect of BDF 9148 was promptly reversed by the sodium channel inactivator tetrodotoxin, an increased sodium influx seems to be the most likely explanation for the drug-induced increase of contractility. Recent measurements of the sodium current in rat isolated myocytes have confirmed this mechanism of action (Honerjäger *et al.*, 1990). The leftward shift of the concentration-response curve of ouabain produced by BDF 9148 fits into the picture because an inhibition of the Na^+/K^+ -ATPase should have a greater effect when the sodium load of the cells is increased. Similar interactions with cardiac glycosides have been observed with DPI 201-106 (Scholtysik *et al.*, 1989) and veratridine (Honerjäger & Reiter 1975).

BDF 9148 and DPI 201-106 prolonged the action potential duration at all stages of repolarization. For DPI 201-106, this is a well known effect (Buggisch *et al.*, 1985; Scholtysik *et al.*, 1985; Kohlhardt *et al.*, 1987; Kihara *et al.*, 1989) which is best explained by a slowed inactivation of the inward sodium current. The increase in action potential duration probably accounts for the associated prolongation of the functional refractory period. In comparison to some ceveratrum alkaloids, which cause an excessive prolongation of the terminal repolarization phase (Honerjäger, 1982), the overall effect of BDF 9148 and DPI 201-106 on the action potential duration is small. Wang *et al.* (1990) have shown that the enantiomers of DPI 201-106 have opposite effects on the sodium channel. (S)-DPI increases the peak inward current and slows its inactivation while (R)-DPI reduces the peak current. Thus the maximum effect of racemic DPI, which was used in our experiments, is limited by a kind of auto-inhibitory mechanism, which may explain the low arrhythmogenicity of this drug. A similar mechanism could limit the action potential prolongation by racemic BDF 9148 (Honerjäger *et al.*, 1990).

BDF 9148 is very lipophilic and only sparingly soluble in water. In aqueous solution, the maximum concentration that can be achieved is about $3 \times 10^{-5} \text{ mol l}^{-1}$. Due to its physicochemical properties, the drug is quickly accumulated in heart tissues. Steady-state tissue concentrations were established within a few minutes. The pharmacological effects of the drug, on the other hand, took much longer to develop. This may indicate that the overall tissue concentration does not reflect the concentration at the pharmacologically relevant binding site in the sodium channel. It was almost impossible to remove BDF 9148 from the atria by washout, which explains the unusual persistence of the drug effects. Effects of BDF 9148 that had been reversed by tetrodotoxin reappeared promptly when both drugs were removed from the bathing fluid, obviously because the water soluble tetrodotoxin was washed away from the sodium channel while the lipophilic BDF 9148 persisted. A similar observation has been made with DPI 201-106 (Buggisch *et al.*, 1985), which is also very lipophilic.

There was one major difference between BDF 9148 and DPI 201-106: while the former had only a small effect on the beating frequency of right atria, a concentration-dependent negative chronotropic effect was seen with DPI 201-106. This drug binds to vascular (Hof & Hof, 1985) and cardiac (Holck & Osterrieder, 1988; Siegl *et al.*, 1988) calcium channels and reduces the slow inward current in slow response action potentials (Buggisch *et al.*, 1985). Thus, calcium antagonism is a probable explanation for the negative chronotropic effect of DPI 201-106. It may also be the cause of the slight reduction of the atrial rate that was observed with the higher concentration of BDF 9148 in our experiments. The finding that beyond $10^{-7} \text{ mol l}^{-1}$, BDF 9148 reduces the calcium current in myocytes (Pfeifer & Ravens, 1990) points in this direction. If this explanation is accepted, a comparison of the negative chronotropic effects of DPI 201-106 and BDF 9148 suggests that the calcium antagonistic properties of the latter are much

weaker than those of the parent compound. However, before a detailed comparison of the influence of both drugs on the cardiac calcium channels has been made, such an assumption remains speculative.

We observed only an increase of the action potential duration in the presence of BDF 9148, while Armah *et al.* (1990a,b) have described a biphasic effect, i.e. a transient prolongation followed by a gradual shortening. The discrepancy may be due, at least in part, to methodological differences. While we increased the concentration of BDF 9148 at 20 min intervals, Armah *et al.* (1990a,b) observed the effect of a single concentration for 90 min. In their experiments action potential shortening usually began after 30–60 min of drug exposure. Thus, even the 1 h observation period with 10^{-5} mol l⁻¹ BDF 9148 in our experiments may have been too short for the development of a biphasic drug effect. However, if the increase of the action potential duration is indeed transient *in vivo*, this could

explain why no increase of the Q–T interval of the ECG is seen in the presence of BDF 9148 (Muster & Raap 1990) while DPI 201-106 usually causes a prolongation (Ruegg & Nuesch, 1987; Kostis *et al.*, 1987).

To sum up, BDF 9148 is an inotropic compound structurally related to DPI 201-106. Our results confirm and extend earlier findings which support the hypothesis that both drugs increase the contractile force via a prolongation of the open state of the sodium channel. BDF 9148 differs from its parent compound mainly by a much weaker influence on the spontaneous beating frequency. The drug may be beneficial in the treatment of congestive heart failure and its *in vitro* and *in vivo* effects certainly deserve further investigation.

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