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Effects of BDF 9198 on action potentials and ionic currents from guinea-pig isolated ventricular myocytes

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1 BDF 9198 (a congener of DPI 201–106 and BDF 9148) was found to be a positive inotrope on guinea-pig isolated ventricular muscle strips. The effects of BDF 9198 on action potentials and ionic currents from guinea-pig isolated ventricular myocytes were studied using the whole cell patch clamp method.

2 In normal external solution, at 37°C, action potential duration at 50% repolarization (APD_{50}) was 167.4±8.36 ms ($n=37$). BDF 9198 produced a concentration-dependent increase in APD_{50} (no significant increase at 1×10^{-10} M; and APD_{50} values of 273.03±35.8 ms at 1×10^{-9} M; $n=6$, $P<0.01$ and 694.7±86.3 ms at 1×10^{-7} M; $P<0.001$, $n=7$). At higher concentrations in the range tested, BDF 9198 also induced early and delayed and after-depolarizations.

3 Qualitative measurements of I_{Na} with physiological $[Na]_o$ showed prolongation of the current by BDF 9198, and the appearance of transient oscillatory inward currents at high concentrations.

4 Quantitative recording conditions for I_{Na} were established using low external [Na] and by making measurements at room temperature. The current–voltage relation, activation parameters and time-course of I_{Na} were similar before and after a partial blocking dose of Tetrodotoxin (TTX, 1 μM), despite a 2 fold difference in current amplitude. This suggests that voltage-clamp during flow of I_{Na} was adequately maintained under our conditions.

5 Selective measurements of I_{Na} at room temperature showed that BDF 9198 induced a concentration-dependent, sustained component of I_{Na} (I_{Late}) and caused a slight left-ward shift in the current–voltage relation for peak current. The drug-induced I_{Late} showed a similar voltage dependence to peak current in the presence of BDF 9198. Both peak current and I_{Late} were abolished by 30 μM TTX and were sensitive to external [Na].

6 Inactivation of control I_{Na} during a 200 ms test pulse to −30 mV followed a bi-exponential time-course. In addition to inducing a sustained current component, BDF 9198 left the magnitude of the fast inactivation time-constant unchanged, but increased the magnitude of the slow inactivation time-constant. Additional experiments with a longer pulse (1 s) raised the possibility that in the presence of BDF 9198, I_{Na} inactivation may be comprised of more than two phases.

7 No significant effects of 1×10^{-6} M BDF 9198 were observed on the L-type calcium current, or delayed and inward rectifying potassium currents measured at 37°C.

8 It is concluded that the prolongation of APD_{50} by BDF 9198 resulted from selective modulation of I_{Na}. Reduced current inactivation induced a persistent I_{Na}, increasing the net depolarizing current during the action potential. This action of the drug indicates a potential for 'QT prolongation' of the ECG. The observation of after-depolarizations suggests a potential for proarrhythmia at some drug concentrations.

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Abbreviations: A_f, inactivating current described by the fast time constant; ANOVA, analysis of variance; APD_{50}, action potential duration at 50% repolarization; APs, action potentials; A_s, inactivating current described by the slow time constant; α subunit, (sodium channel) alpha-subunit; BAPTA, 1.2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid; BDF 9148, (4-(3-(1-diphenylmethyl-azetidine-3-oxy)-2-hydroxypropoxy)-1-H-indol-2-carbonitrile; BDF 9198, (4-(3-(1-diphenylmethyl-azetidine-3-oxy)-2-hydroxy-propylamin)-1-H-indol-2-carbonitrile; C, residual current at end of voltage pulse; Ca_i, internal calcium transient; Ctl, control; DADs, delayed after-depolarizations; DMSO, dimethylsulphoxide; DPI-201-106, 4-[3-(4-diphenylmethyl-1-piperazinyl)-2-hydroxypropoxy]-1H-indole-2-carbonitrile; EADs, early after-depolarizations; G_{max}, maximal I_{Na} conductance; h1, human cardiac sodium channel; I, tetrodotoxin sensitive cardiac sodium channel; $I_{Ca,L}$, L-type calcium current; IClamp, current clamp; I_K, delayed rectifier potassium current; I_{Kl}, inward rectifier potassium current; I_{Late}, sustained current remaining at end of voltage pulse; I_{Na}, sodium current; I_{Peak}, peak current at start of voltage pulse; I_{ti}, transient inward current; I-V, current–voltage relation; k, slope factor; LJP, liquid junction potential; LQTS, long QT syndrome; $[Na]_I$, Internal sodium concentration; $[Na]_o$, external sodium concentration; NMDG, N-methyl-D-glucamine; s.e.m., standard error of the mean; τ_f, fast time constant; τ_s, slow time constant; TTP, time to peak; TTX, tetrodotoxin; TTX-S, tetrodotoxin-subtraction; $V_{0.5}$, membrane potential exhibiting half maximal current activation; V_m, membrane test potential; V_{rev}, reversal potential

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Introduction

Although positive inotropes are a cornerstone in the treatment of heart failure, the inadequacy of the agents currently used in clinical practice is well recognised and has provided the impetus for the continuing development of new approaches (Dorigo *et al*., 1994). Prolongation of the action potential is often associated with a positive inotropic effect. One possible approach to the development of a new positive inotrope, therefore, is the synthesis of agents that prolong the action potential by inhibiting the closing of cardiac sodium channels.

Molecular biology techniques have separated five sodium channels, two of which exist in cardiac muscle, the I and h1 channels, which exhibit differential sensitivities to tetrodotoxin (TTX; e.g. Watson & Girdlestone, 1995). The opening of the TTX-resistant h1 sodium channel on cardiac muscle, which is blocked by μM TTX, produces the rapid depolarization (phase 0) of the cardiac action potential. The opening of this sodium channel in turn leads to the opening of voltage-dependent calcium channels and ensuing entry of calcium into the cell triggering calcium release from sarcoplasmic reticulum. Increasing intracellular Ca^{2+} concentrations leads to an increase in the force of contraction. The function of the second sodium channel in the heart, the TTX-sensitive I channel which is blocked by nM TTX, is unknown.

Low concentrations of the naturally occurring alkaloid veratridine prolong the action potential by preventing closure of the Na channel inactivation gate (Honerjager, 1982) and have marked positive inotropic effects. Higher concentrations of veratridine, however, also open Na channels (Honerjager, 1982) and can produce after-depolarizations, ectopic beats and arrhythmias (Doggrell *et al*., 1995; Nand *et al*., 1997). Veratridine also prolongs the opening of the neuronal Tetrodotoxin-sensitive I channel to promote neurotransmitter release (Honerjager, 1982). Veratridine is unsuitable as a therapeutic inotrope because of the cardiotoxicity and lack of cardioselectivity.

Cardioselective inhibitors of the TTX-resistant h1 Na channel inactivation have also been developed. The most widely studied of these are DPI 201-106 and its congener BDF 9148 (Ravens *et al*., 1995; Doggrell & Brown, 1997). These studies have confirmed that prolongation of the opening of the sodium channel with DPI 201-106 or BDF 9148 is associated with a prolongation of the action potential and a positive inotropic effect (Ravens *et al*., 1995; Doggrell & Brown, 1997). The ion channel modulatory effects of DPI 201-106 and BDF 9148 are not limited to sodium channels, however. Both DPI 210-106 and BDF 9148 inhibit the L-type calcium current (Ravens *et al*., 1991; 1995), and DPI 210-106 can inhibit inward and delayed rectifier potassium currents (Amos & Ravens, 1994). Animal studies suggest that DPI 201-106 may be proarrhythmic (Novosel *et al*., 1993). Poor bioavailability made BDF 9148 unsuitable for clinical trial and it has been superseded by BDF 9198 which has improved bioavailability (Doggrell & Brown, 1997).

The positive inotropic effect of BDF 9198 has been demonstrated on isolated human myocardium (Muller-Ehmsen *et al*., 1998; Schwinger *et al*., 1999). However there have been no reports characterizing the effects of BDF 9198 on action potentials, or sodium and other channels. In this study we report that BDF 9198 exerts a positive inotropic effect on ventricular muscle strips isolated from the guinea-pig. We have characterized the effects of BDF 9198 on ventricular action potentials (APs) from isolated guinea-pig ventricular myocytes and report AP prolongation. A major aim of our study was to investigate the effects of BDF 9198 on ionic currents from ventricular myocytes. We report modulation by BDF 9198 of the fast sodium current (I_{Na}) and also investigated the drug's effects on the L-type calcium current, and the inward and outward rectifying potassium currents.

Methods

Ventricular strip contractility

Guinea-pigs were stunned and exsanguinated. The heart was rapidly removed and placed in Krebs solution that was saturated with 5% CO_2 in oxygen at 37°C, and the free walls of the right and left ventricles were excised. All experiments were performed in the presence of a modified Krebs solution [composition (mM): NaCl, 116; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 22.0; D-glucose, 11.2] containing guanethidine at 10^{-5} M to prevent the release of noradrenaline from nerve endings, and atropine at 10^{-6} M to block muscarinic receptors at 37°C. We have previously described the method for recording ventricular contractility (Nand & Doggrell, 1999). Strips were prepared from the ventricle free wall and mounted longitudinally between two platinum electrodes under 1 g tension, in 5 ml organ baths in Krebs solution being vigorously bubbled with 5% CO_2 in oxygen and allowed to equilibrate for 75 min. Contractile responses were measured isometrically with force displacement transducers (Grass model FT03.C) and displayed on a polygraph (Grass model 79B). After stimulation at 4 Hz (5 ms duration, 10 V) for 6 min, BDF 9198 at 10^{-8} M was added. The cumulative addition of BDF 9198 (3×10^{-8}, 10^{-7} M etc.) occurred on a 5 min cycle until conclusion of the experiment at a concentration of 1×10^{-6} M. The augmentation of the contractile response in the presence of each concentration of BDF 9198 was calculated as per cent change and mean values $\pm$ s.e.m. were determined.

Myocyte isolation

Myocytes were isolated from both ventricles of male guinea-pig ($\sim$400 g) hearts, using an enzymatic dispersion method described previously (Levi & Issberner, 1996). The cells were stored at room temperature in solution containing 1 mM Ca^{2+} until required for use. Cells remained viable for up to 8 h after isolation.

Electrophysiology

Isolated cells were placed in a Perspex chamber mounted on an inverted microscope (Nikon Diaphot 300), allowed to settle, and then superfused at 37°C with a Tyrode's solution containing (in mM): NaCl 140; KCl 4, $CaCl_2$ 2.5; $MgCl_2$ 1; Glucose 10; HEPES 5 (adjusted to 7.45 with NaOH). All experiments were carried out at 37°C, with the exception of selective I_{Na} recordings which were conducted at room temperature (20–22°C). External superfusate was changed using a rapid solution-switching device (Levi *et al*., 1996). Patch pipettes (Corning 7052 glass, AM Systems, Everett, U.S.A.) were pulled (Puller, Model P-87, Sutter Instrument, U.S.A.) and polished (Narishige MF-83 microforge) to resistances of between 2 and 3 MΩ in all experiments except for the selective I_{Na} recordings. For these, pipettes had resistances of between 1 and 2 MΩ. Patch pipette filling

solutions and external solutions are detailed in Table 1 and recording conditions summarized below.

As I_{Na} is large and fast, special conditions were necessary in order to record it quantitatively. Thus these experiments were conducted at room temperature in order to slow the kinetics of the current. Low resistance patch-pipettes were used to facilitate intracellular dialysis of cells with the pipette solution and to minimize voltage errors due to uncompensated series resistance. Caesium-based internal and external experimental solutions containing similar levels of sodium were used to reduce the gradient for sodium entry (thereby reducing the size of the current), and to block any contamination from potassium currents. Nifedipine was used externally to block interfering calcium current. I_{Na} was measured selectively as the difference current following application of TTX (30 μM). For the solutions used for selective sodium current recording described in Table 1 the liquid junction potential (LJP) between the pipette and the external solution was -1.06 ± 0.33 mV ($n=6$ pipettes; mean $\pm$ s.e.m). Due to the small size of the LJP value, no corrections to the data were made.

Delayed rectifier (I_K) and inward rectifier (I_{K1}) currents were recorded using a potassium based sodium-free pipette solution, and N-methyl-D-glucamine (NMDG) externally. Replacing the external sodium with NMDG inhibited contaminating inward Na-Ca exchange tail currents that can otherwise occur on repolarization from positive test potentials. Nitrendipine or nifedipine were used externally to block interfering calcium current. $BaCl_2$ was included in external solutions for recording I_K to inhibit any interference from I_{K1}. L-type calcium current ($I_{Ca,L}$) was recorded using a Cs-based pipette solution to inhibit potassium current interference, and an external NMDG solution. In all voltage clamp experiments, 1.2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA, K salt) was included in the pipette filling solutions to inhibit interfering internal Ca (Ca_i)-activated currents. Current clamp (I Clamp) and standard voltage clamp experiments (qualitative measurements of I_{Na}) were conducted using standard internal and external solutions (Table 1).

Whole cell voltage-clamp recordings were made using an Axopatch 200 amplifier (Axon Instruments, California, U.S.A.) with a CV-201 head-stage, and whole cell current-clamp recordings were made using an Axopatch 1D amplifier with a CV-4 head-stage. Cell capacitance was measured by either analysing the charging transients elicited by a 5 mV voltage step or reading the capacitance value from the dial on the Axopatch amplifier after compensating for series resistance and cell capacitance. These methods have been shown previously to give similar values for cell capacitance (Hancox *et al*., 1993). Series resistance values were in the range of 2–4 MΩ; for six cells a mean value of 3.08 ± 0.35 MΩ was obtained. Typically, 75–80% of series resistance could be compensated. Voltage errors during recordings of peak I_{Na} were estimated for six cells and ranged between 1.5 and 4 mV (mean $= 2.29 \pm 0.38$ mV). The measurement of I_{Na} as difference current sensitive to 30 μM TTX in most experiments minimized the likelihood that current records were contaminated with residual uncompensated capacitative current.

Data analysis and statistics

Voltage and current clamp protocols were generated using the program Winwcp (version 1.7a; written and supplied by John Dempster of Strathclyde University, U.K.) *via* a CED (Cambridge Electronic Design, U.K.) 1401 digital interface or a Digidata 1200B interface (Axon Instruments, U.K.). Data

Table 1 Composition of internal and external solutions used in whole-cell recordings

*Standard voltage clamp & I clamp**															
Internal			*External*			*Internal*			*External*			*Internal*			*External*		
KCl	113	KCl	4	130	CsCl	130	CsCl	CsCl	113	KCl	4	KCl	113	KCl	4		
$MgCl_2$	0.4	$MgCl_2$	1	0.4	$MgCl_2$	1.2	$MgCl_2$	$MgCl_2$	0.4	$MgCl_2$	1	$MgCl_2$	0.4	$MgCl_2$	1		
K_2ATP	5			5	MgATP			MgATP	5			K_2ATP	5				
HEPES	10	HEPES	5	10	HEPES	20	HEPES	HEPES	10	HEPES	5	HEPES	5	HEPES	5		
Glucose	5	Glucose	10	5	Glucose	11	Glucose	Glucose	5	Glucose	10	Glucose	10	Glucose	10		
BAPTA*	5			5	BAPTA			BAPTA	5			BAPTA	5				
NaCl	10	NaCl	140	10	NaCl	10	NaCl										
		$CaCl_2$	2.5			1	$CaCl_2$			$CaCl_2$	2.5					$CaCl_2$	2.5
										NMDG	140					NMDG	140
						0.02	Nifedipine									Nitrendipine	0.02
						0.03	TTX‡									$BaCl_2$‡	0.1
pH 7.2		pH 7.45			pH 7.3		pH 7.3	pH 7.2		pH 7.45		pH 7.2		pH 7.45			
KOH		NaOH			CsOH		CsOH	CsOH		HCl		CsOH		HCl			

All concentrations in mM. ◆*Substance omitted from experimental solutions. ‡Added after recording I_{Na} for current subtraction purposes.

were recorded on-line at 2 kHz, except I_{Na} data for which a recording frequency of 10 kHz was used. Data were stored on the hard disk of an IBM compatible PC, and analysed using Winwcp. Figures were constructed using FigP (Biosoft), and statistical analysis performed using EXCEL (Microsoft). Data are shown as mean ± s.e.m. and were compared statistically using Students' *t*-test or ANOVA with a *post-hoc* Bonferroni correction. A P value of <0.05 was taken as significant.

Chemicals and drugs

BDF 9198 (4-(3-(1-diphenylmethyl-azetidine-3-oxy)-2-hydroxy-propylamin)-1-H-indol-2-carbonitrile (donated by Beiersdorf–Lilly, Hamburg, Germany) was made as a 1 mM stock solution in ethanol, serially diluted using deionized water (Milli-Q, Millipore, U.S.A.), and then aliquoted and stored at −20°C. It was added to the experimental solutions to give final concentrations between 1×10^{-10} and 1×10^{-5} M. All isolation solutions were made from Aristar grade chemicals, supplied by British Drugs House (BDH). Type 1A collagenase was supplied by Worthington, U.S.A. and Type XIV protease was supplied by Sigma. Analar grade chemicals (BDH) were dissolved in deionized water to make external solutions, and Aristar grade chemicals (BDH) were used to make internal solutions. NMDG (Sigma) replaced NaCl in the sodium-free external solution. Nitrendipine (Research Biochemicals International) and Nifedipine (Sigma) were dissolved in dimethylsulphoxide (DMSO) to give 10 mM stock solutions. Both were shielded from light as they are light sensitive. Barium chloride ($BaCl_2$) was dissolved in deionized water to give a stock solution of 1 M. Tetrodotoxin (TTX) obtained from Tocris was made as a 3 mM stock solution in deionized water. Aliquots of nitrendipine, nifedipine, $BaCl_2$ and TTX were added to the appropriate test solutions to give the final concentrations shown in Table 1.

Results

Effects of BDF 9198 on contractility

Before investigating effects of BDF 9198 on electrophysiological properties of guinea-pig myocytes, we first determined whether or not the compound was positively inotropic on ventricular tissue from this species. Electrically stimulated contractions of left and right ventricular muscle strips were measured as described in Methods and concentrations of BDF 9198 between 1×10^{-8} and 1×10^{-6} M were applied. The drug produced a concentration-dependent augmentation of isometric force generation (Figure 1). The augmentation was similar between strips from left and right ventricles, and was maximal at 3×10^{-7} M (Figure 1; there was no significant difference between per cent augmentation at 3×10^{-7} and 1×10^{-6} M).

Effects of BDF 9198 on action potential duration (APD).

Ventricular action potentials were recorded in 'current clamp' mode, with internal and external solutions as described in Table 1. Brief (<10 ms) suprathreshold current pulses were applied every 3 s, and action potentials recorded in normal Tyrode's solution and after addition of BDF 9198. APD was measured at 50% repolarization (APD_{50}). In normal Tyrode's solution the mean APD_{50} was 167.4 ± 8.36 ms ($n=37$ cells) and

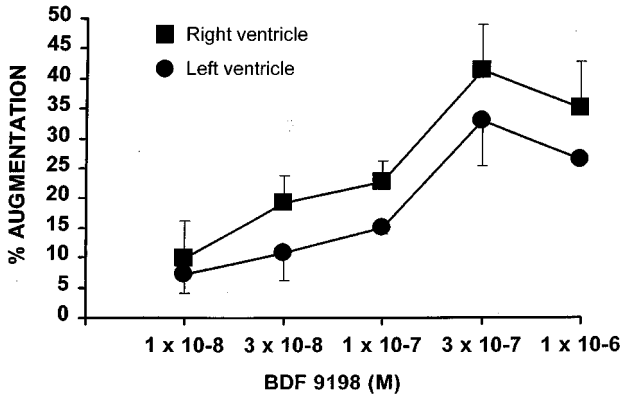

Figure 1 Effect of BDF 9198 on muscle strip contractility. Percentage (%) augmentation of the electrical stimulation force–responses of right and left ventricular muscle strips produced by a range of concentrations of BDF 9198. Per cent augmentation is plotted against the logarithm of the molar concentration of BDF 9198. Each value is the mean ± s.e.m. from five strips from separate guinea-pigs.

external application of BDF 9198 produced a dose-dependent action potential lengthening (Figure 2). For a given drug concentration the effect on APD reached a steady-state within ~2–3 min of application to the cell under study. 1×10^{-10} M caused no significant lengthening of the action potential. At 1×10^{-9} M there was an increase in the APD_{50} (to 273.03 ± 35.84, $n=6$; $P<0.01$ compared to control). 1×10^{-8} M BDF 9198 increased the APD_{50} to 440.6 ± 53.3 ms ($n=6$; $P<0.001$ compared to control). At 1×10^{-7} M, BDF 9198 caused a profound lengthening of the action potential plateau, increasing the APD_{50} to 694.7 ± 86.3 ms ($n=7$; $P<0.001$). At the higher concentration of 1×10^{-6} M, the AP duration was increased further. However the degree of prolongation at this concentration was such that cells started to round up before steady state APD_{50} values could be reliably made.

BDF 9198 was also observed to induce depolarizations which occurred prior to, and following action potential repolarization, and are therefore classifiable as 'early after-depolarizations' (EADs) and 'delayed' after-depolarizations (DADs; Ferrier, 1977; January & Fozzard, 1988). These were observed after cells had been exposed to the drug for periods of ~ 4 min or longer. At 1×10^{-7} M, four of the cells exhibited DADs with prolonged BDF 9198 exposure, one exhibited EADs, one exhibited both DADs and EADs, and one showed neither.

The action potential prolongation observed in Figure 2 could have resulted from the drug enhancing inward (depolarizing) currents during the action potential plateau phase, reducing outward (repolarizing) currents, or a combination of these actions. Therefore, we investigated the effects of BDF 9198 on major inward and outward current systems, under whole cell voltage clamp.

Effect of BDF 9198 on I_{Na}

It is widely accepted that, with normal levels of external Na, and at a physiological temperature, cardiac sodium current is difficult to voltage-clamp adequately, due to the size and speed of the current. Nonetheless, we regarded it worthwhile in initial experiments to determine qualitatively the effects of BDF 9198 on I_{Na} measured with standard internal and external solutions. Figure 3a shows a typical I_{Na} elicited by a 250 ms duration voltage step from −80 to −40 mV. Under control conditions a large (off-scale) rapidly activating and inactivating current

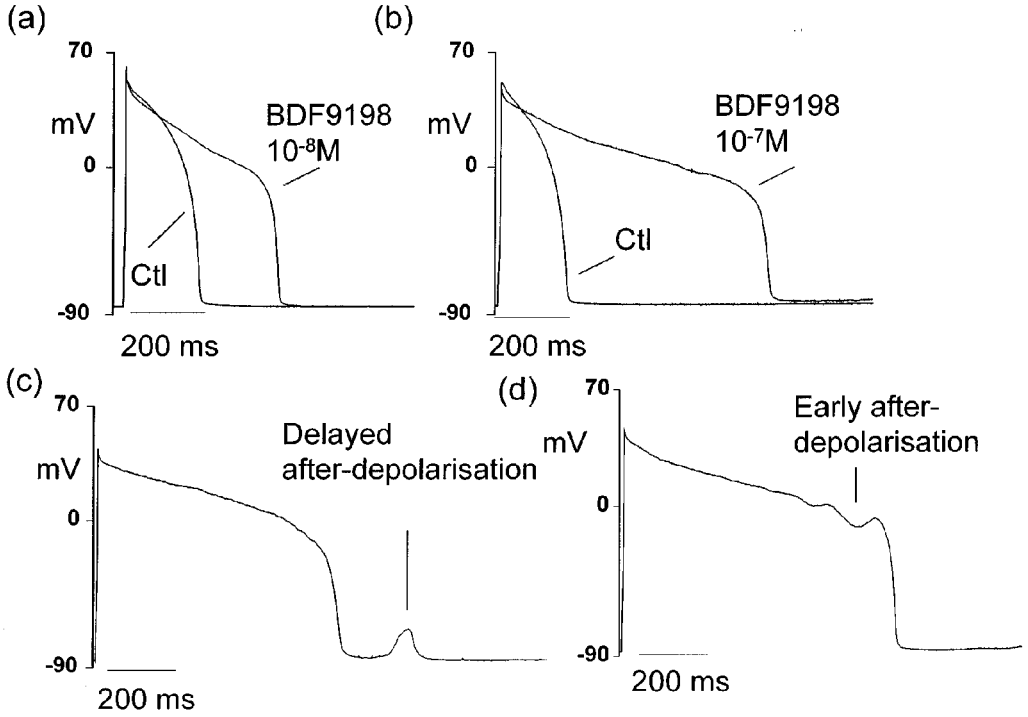

Figure 2 Effect of BDF 9198 on action potentials. Action potentials were recorded in 'current clamp' mode. (a) Action potential with normal external Tyrode's solution (Ctl) and following exposure to 1×10^{-8} M BDF 9198. (b) In a different cell, the effect of 1×10^{-7} M BDF 9198. The drug produced a dose-dependent prolongation of the action potential. (c) An action potential from another cell in the presence of 1×10^{-7} M BDF 9198, followed by a delayed 'after-depolarization'. (d) From another cell, an 'early after-depolarization recorded in the presence of 1×10^{-7} M BDF 9198.

was elicited (left panel). After application of BDF 9198 at 1×10^{-6} M (right panel) there was a clear increase in the duration of the current, with the current profile appearing similar to an inverted action potential (and saturating the voltage clamp). This current profile shared similarities with that reported previously for the related compound BDF 9148 (Ravens *et al*., 1991). The prolonged I_{Na} was followed by a subsequent oscillatory current which resembles an I_{ti} (Lederer & Tsien, 1976; Eisner & Lederer, 1979), and is consistent with proarrhythmic potential for the drug suggested by the action potential recordings.

Establishing selective recording conditions for I_{Na}

In order to investigate the effects of BDF 9198 on I_{Na} in a selective and quantitative manner, it was necessary to establish conditions that would permit accurate whole cell measurements of I_{Na}. For these experiments solutions were chosen (see Table 1) that eliminate interfering currents and reduce the amplitude of the current (by reducing $[Na]_o$ to 10 mM). Measurements were conducted at room temperature (20–22°C) in order to slow I_{Na} kinetics. Low resistance pipettes were used to minimise potential voltage errors which might arise during flow of I_{Na} (see Methods for estimated values).

Previous studies (e.g. Makielski *et al*., 1987; Miyamoto *et al*., 1991; Feng *et al*., 1996) have reported a negative shift in the voltage-dependent inactivation kinetics of I_{Na} under conditions of internal fibre/cell dialysis. This necessitates applying depolarizing test-pulses from a highly negative membrane potential in order to avoid partial inactivation of I_{Na}. In our I_{Na} experiments, therefore, whilst cell membrane potential was held at −80 mV, a 2 s pre-pulse to −140 mV preceded each depolarizing test pulse.

Figure 3b (left panel) shows sample currents elicited by step depolarizations from −140 mV to −50, −40 and −30 mV. Application of 30 μM TTX completely eliminated the observed inward currents and subtraction of control recordings from those in the presence of TTX provided 'TTX-subtraction' current measurements (TTX-S; Figure 3b right panel). TTX-S and control currents were very similar, suggesting that we were successful in measuring I_{Na}. This is further highlighted by close similarity in the current–voltage (I–V) relations for the control and TTX-S currents shown in Figure 3c (example from one cell). Peak I_{Na} values between 3 and 5 nA were observed under these recording conditions and I–V relations for individual cells crossed the voltage axis between 0 and +12 mV.

Previously, experiments in which the I–V relations for I_{Na} were compared between 'control' and partially inhibited current have been used to assess the quality of the voltage clamp during flow of I_{Na} (e.g. Brown *et al*., 1981; Follmer *et al*., 1987). We used an approach similar to that described by Follmer *et al*: the effects were determined of a partial blocking dose (1 μM) of TTX on the I–V relation for, and activation and inactivation time-courses of I_{Na}. Figure 4a shows recordings of I_{Na} elicited by a step-depolarization from a prepulse potential of −140 to −30 mV, in the presence and absence of TTX. 1 μM TTX (middle panel) reduced the peak I_{Na} to less than 50% of the control current (left panel). A higher dose of 30 μM TTX, abolished the I_{Na} completely (right panel). The currents obtained in control conditions and after the partial blocking dose of 1 μM TTX were subtracted from those in the presence of 30 μM TTX, to give TTX-S I_{Na} from which I–V relations were constructed. The mean I–V relations for TTX-S control current and current sensitive to 1 μM TTX are shown in Figure 4b. Each data-set was fitted by a modified Boltzmann equation of the form:

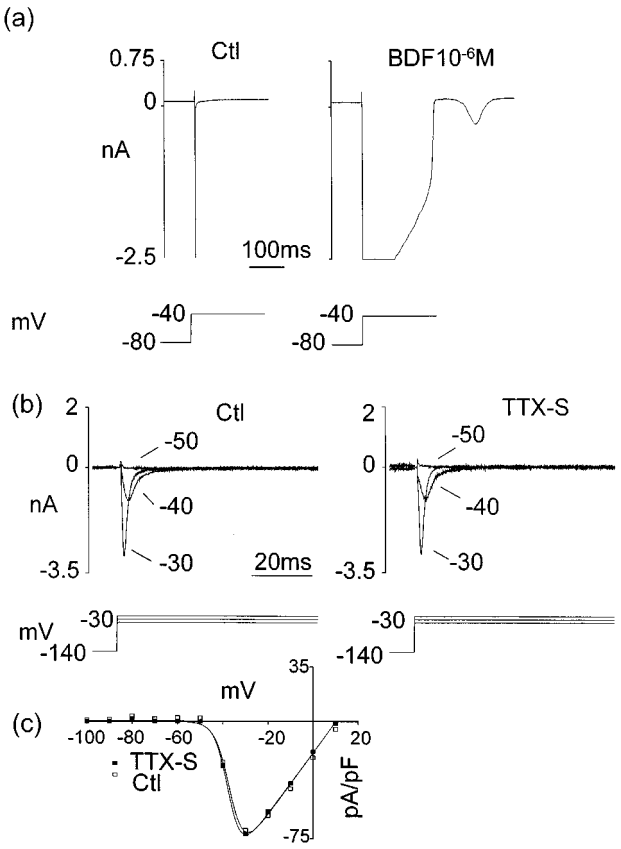

Figure 3 Effect of BDF 9198 on I_{Na} with normal $[Na]_o$ and establishment of selective I_{Na} recording conditions with reduced $[Na]_o$. (a) Recordings of I_{Na} (upper traces) using standard Tyrode's solution externally, and a potassium based pipette filling solution. Lower traces show voltage protocol (250 ms step from -80 to -40 mV). BDF 9198 produced an increase in the duration of I_{Na}. Note that in the record showing effects of 10^{-6} M BDF 9198, an oscillatory inward current followed the large (poorly clamped) I_{Na}. (b) Establishment of selective I_{Na} recording conditions (see Methods). Left hand panel (Ctl) shows currents (upper traces) activated on step depolarization from -140 mV to -50, -40 and -30 mV (lower traces). In the same cell, TTX (30 μM) was applied and the TTX-subtraction (TTX-S) currents are shown in the right panel. (c) The I–V relation shows close similarity between Ctl and TTX-S currents.

$$I_{Na} = G_{max}(V_m - V_{rev})/(1 + \exp[(V_{0.5} - V_m)/k]) \quad (1)$$

where I_{Na} represents current density at test potential V_m, G_{max} is maximal I_{Na} conductance, V_{rev} is the reversal potential, $V_{0.5}$ is the membrane potential exhibiting half maximal current activation, and k is the slope factor which describes the steepness of activation for the current. The Boltzmann fit to the data gave a value for $V_{0.5}$ of -39.4 ± 0.49 mV and $k=4.92\pm0.42$ mV in control solution. This was not significantly different after the application of 1 μM TTX, where $V_{0.5}=-40.7\pm0.45$ mV and $k=4.4\pm0.30$ mV in six cells ($P>0.05$). Thus, it was evident that although 1 μM TTX reduced the magnitude of current at all test potentials, there was no significant effect on the activation parameters derived from the curve-fit to the I–V data. Furthermore, our observed $V_{0.5}$ values compare favourably to that reported previously for human atrial I_{Na} (-38.6 mV; Feng *et al*., 1996) and the mean voltage for half maximal Na conductance reported for cat atrial I_{Na} (-41.8 mV) by Follmer *et al*. (1987).

To quantify the time-course of activation, we measured the time to peak (TTP) I_{Na}, over a range of test potentials. TTP was measured from the start of the voltage step to the peak of the current. Under control conditions, selected values obtained were (in ms): -40 mV: 1.43 ± 0.18; -30 mV: 1 ± 0.13; -20 mV: 0.70 ± 0.11; -10 mV: 0.46 ± 0.12. These were similar to other reported values (for a review see Fozzard & Hanck, 1996). The values for TTP after application of 1 μM TTX were not significantly different from those under control conditions (-40 mV: 1.69 ± 0.23; -30 mV: 1.14 ± 0.15; -20 mV: 0.73 ± 0.05; -10 mV: 0.40 ± 0.06; $P>0.4$ at each potential).

Under our recording conditions we found that control I_{Na} inactivated very rapidly, and the time-course of inactivation was well described by a bi-exponential process. We were able to quantify the time-course of inactivation by fitting the I_{Na} records with the following equation:

$$A_f \times \exp(-x/\tau_f) + A_s \times \exp(-x/\tau_s) + C \quad (2)$$

where A_f is the current described by τ_f and A_s the current described by τ_s, and where C represents any residual current component remaining at the end of the pulse, fitted by neither time-constant (under our conditions, $C=0$ for I_{Na} in the absence of BDF 9198). Under control conditions the values for τ_f were $\sim$4 fold lower than the values for τ_s. Both τ_f and τ_s values showed voltage-dependence, decreasing progressively at more positive test potentials (see Table 2). The relative proportion of current inactivation fitted by each τ value did not significantly alter over the voltage range studied. The time-constant values obtained from currents fitted after the application of the partial blocking dose of TTX (see Table 2), were not significantly different from control values ($P>0.15$ for comparisons at each voltage). Furthermore, our inactivation time-constant values were comparable to other studies in which I_{Na} was recorded using the whole cell patch clamp technique (e.g. Sakakibara *et al*., 1992; Conforti *et al*., 1993), and were comparable with (although smaller than) published data obtained at a lower temperature (10–13°C) using large bore suction pipettes (Hanck & Sheets, 1992).

The similarity between control and partially blocked I_{Na}, of the I–V relations and parameters for both activation and inactivation (despite the large difference in current amplitude) suggested that the quality of the voltage clamp was not compromised under our recording conditions.

One feature of our I_{Na} recording conditions merits further mention. Despite similar [Na] in bulk pipette and external solutions, the mean I–V relations for TTX-S I_{Na} (Figure 4b) intersected the voltage axis positive to 0 mV, the value expected for equimolar [Na]. Therefore, we performed some additional experiments with different internal and external solutions used in prior successful measurements of I_{Na} by Sakakibara *et al*. (1992; CsF based internal solution; TMA-Cl based external solution; 5 mM bulk [Na] in both internal and external solutions). We observed a voltage dependence for I_{Na} with these solutions very similar to that described above ($V_{0.5}=-36.7\pm1.2$ mV; $k=5.5\pm0.7$, $n=5$; $P>0.05$). Furthermore, the mean I–V relationship with these solutions also intersected the voltage axis positive to 0 mV under our conditions (data not shown) but close to 0 mV in the experiments by Sakakibara *et al*. It is possible, therefore, that whilst we were successful in controlling bulk [Na] concentrations sufficiently to permit adequate voltage clamp of I_{Na}, the [Na] close to the cell membrane may not have precisely reflected concentrations in the bulk solution. Taken collectively, however, the data described above very strongly indicate that our experimental conditions permitted sufficiently accurate recordings of I_{Na} voltage-dependence and time-course, to allow quantitative investigation of the effects of BDF 9198 to be undertaken.

Effects of BDF 9198 on I_{Na} measured selectively

Figure 5a shows the effect of 1×10^{-6} M BDF 9198 on I_{Na} elicited by depolarizing test pulses from -140 to -30 mV. Under control conditions (left panel) I_{Na} activated rapidly (reaching a peak of $\sim$4.3 nA) and then inactivated completely within $\sim$20 ms. In the presence of 1×10^{-6} M BDF 9198, I_{Na} was significantly altered, with a profound effect on current inactivation. At the end of the 200 ms long voltage pulse there was still a substantial inward current remaining (middle panel). Application of 30 μM TTX completely abolished the sustained inward current produced by BDF 9198. This also allowed us to use the 'TTX-S' current as a measure of I_{Na} both before and after exposure to BDF 9198. The effect of BDF 9198 on I_{Na} was long-lasting, and was not readily reversible on returning to control solution. This contrasted with the effect of TTX which was readily reversible (within 3 pulses following washout, data not shown).

Table 2.

	Control		*1 μM TTX*	
Test Pot.	τ_f	τ_s	τ_f	τ_s
-40	2.16±0.22	13.66±0.90	2.64±0.30	14.97±0.81
-35	1.73±0.19	10.85±0.88	1.93±0.26	10.45±0.90
-30	1.30±0.07	10.65±0.80	1.43±0.13	10.06±0.74
-25	1.12±0.09	9.51±0.15	1.15±0.12	8.12±0.86
-20	0.84±0.07	7.75±0.98	0.98±0.09	8.17±0.99
-15	0.69±0.08	7.03±0.86	0.81±0.07	7.08±0.70
-10	0.54±0.09	6.28±0.50	0.73±0.08	6.41±0.71
	A_f	A_s	A_f	A_s
-40	87.17±2.63	12.83±2.64	92.01±3.51	7.99±3.52
-35	93.67±1.30	6.33±1.31	93.83±1.72	6.17±1.72
-30	96.83±0.31	3.17±0.31	96.00±0.51	4.00±0.51
-25	97.00±0.68	3.00±0.68	95.67±0.95	4.33±0.95
-20	97.00±0.37	3.00±0.37	96.00±0.68	4.00±0.68
-15	94.50±1.31	5.50±1.31	93.50±1.08	6.50±1.08
-10	92.83±2.86	7.17±2.86	93.67±1.28	6.33±1.28

Figure 5b shows the results obtained in additional experiments performed to confirm that Na was the charge carrier for the sustained current component induced by BDF 9198. Each of five cells were dialysed with Na-free pipette solution and pulses applied from -140 to -30 mV. 1×10^{-6} M BDF 9198 produced a sustained inward current at the end of the pulse (middle panel), compared to the rapidly inactivating current in control (left panel). Subsequent replacement of the external Na with NMDG eliminated both the rapid and sustained current components (right panel). The sensitivity of the sustained inward current to external Na indicates that this current component was carried by flow of Na during an I_{Na}.

Figure 6 shows families of I_{Na} (as TTX-S current; shown on an expanded time-base) elicited by 200 ms duration step depolarizations from -140 mV to a range of test potentials. Under control conditions (Figure 6a) peak I_{Na} in this cell increased in amplitude with test pulse magnitude, up to -35 mV and then decreased with steps to more positive potentials, reversing near 0 mV. In the presence of 1×10^{-6} M BDF 9198 (Figure 6b), significant I_{Na} was observed at potentials at which only small current had been observed in control solution and the maximal peak I_{Na} was observed at -45 mV. These observations were suggestive of a negative shift in the voltage dependence of the current. In addition, at all test potentials, BDF 9198 reduced current inactivation during the pulse, giving rise to a sustained component of I_{Na}, which was absent under control conditions.

The mean effects of BDF 9198 are also shown in Figure 6, which shows I–V plots ($n=6$) for peak current (I_{Peak}; Figure 6c) and end-pulse current (I_{Late}; Figure 6d). Under both control conditions and with BDF 9198, the I–V relation for I_{Peak} was 'bell-shaped' and crossed the voltage axis between 0 and +12 mV (with E_{rev} more negative in the presence of BDF

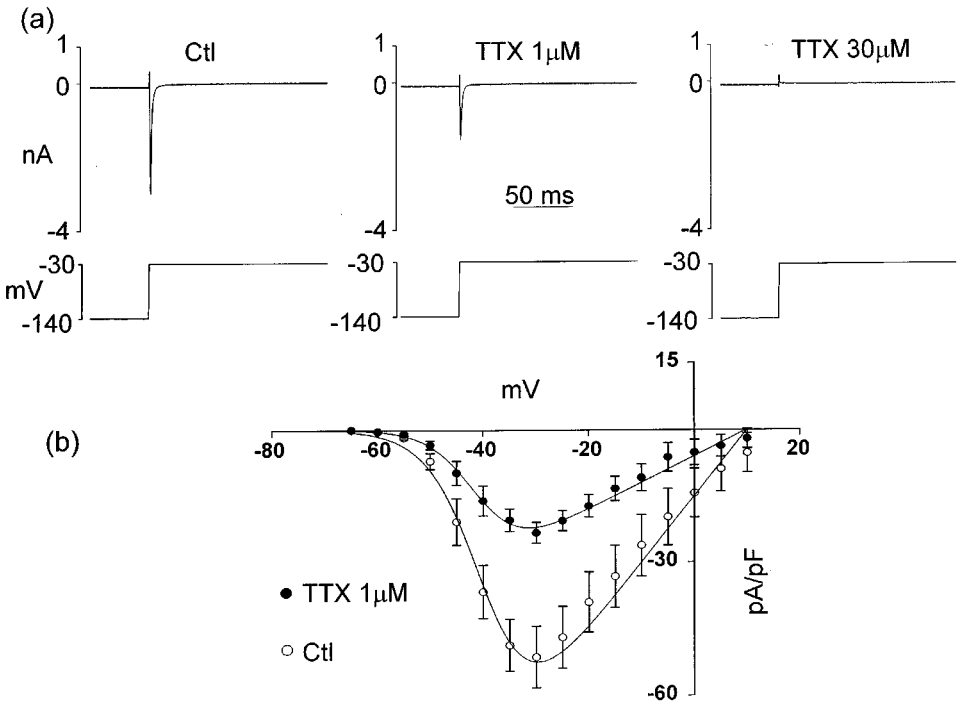

Figure 4 Effects of a partial blocking dose of Tetrodotoxin on I_{Na}. (a) Selective I_{Na} recordings (upper traces) elicited by a step depolarization from -140 mV to a test potential of -30 mV (lower traces). In control conditions (Ctl; left panel) a rapidly activating and inactivating I_{Na} was observed. 1 μM TTX produced a partial block of I_{Na} (middle panel); a higher concentration of 30 μM TTX (right panel) completely abolished I_{Na}. (b) shows the mean current-voltage (I–V) relationship for peak I_{Na} under control conditions and after the application 1 μM TTX. Curve fits to the data were made using equation 1 (Results).

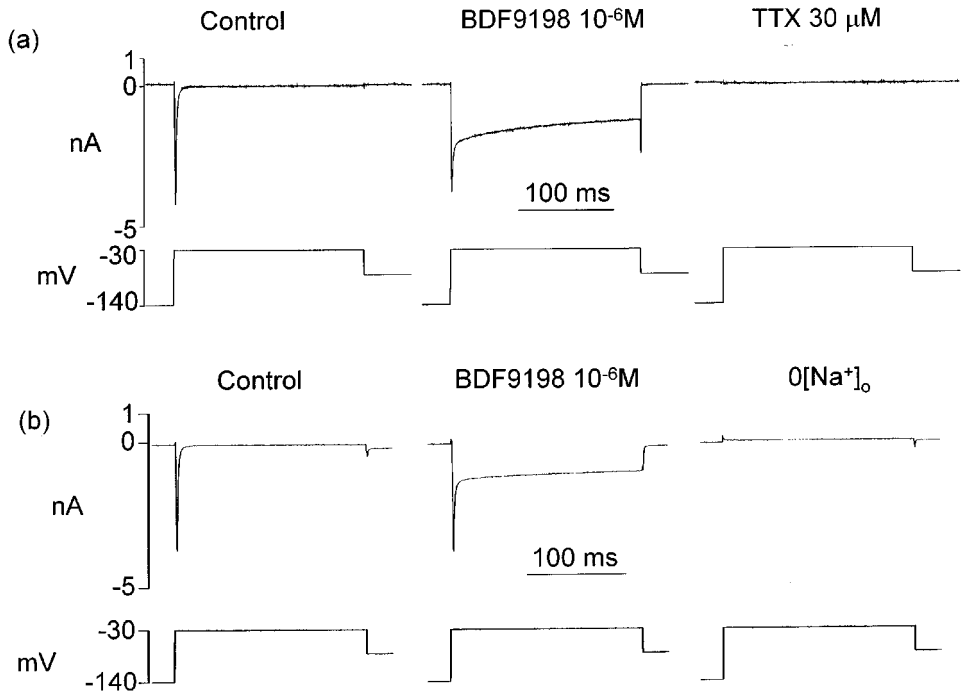

Figure 5 Selective recordings of the effect of BDF 9198 on I_{Na}. (a) shows selective I_{Na} recordings (upper traces) at a test potential of −30 mV (lower traces). In control conditions (left panel) a rapidly activating and inactivating I_{Na} was observed. 1×10^{-6} M BDF 9198 produced a profound prolongation of I_{Na} (middle panel). The current in the presence of BDF 9198 was abolished by 30 μM TTX (right panel). (b) Recordings of I_{Na} (upper traces) from a different cell elicited by a step depolarization from −140 mV to a test potential of −30 mV (lower traces). The current in control conditions (left panel) was prolonged upon application of 1×10^{-6} M BDF 9198 (middle panel). External application of a Na-free solution abolished the current (right panel), confirming its identity as an I_{Na}.

9198, possibly due to raised Na in the subsarcolemmal space, *cf* Convery & Hancox, 1999). The mean (±s.e.m.) data for both plots were fitted with equation 1. Under our conditions, activation of I_{Na} increased steeply over the voltage range between −50 and −30 mV. For I_{Peak} under control conditions (open circles), the Boltzmann fit to the data gave a value for $V_{0.5}$ of −39.0±0.4 mV, and $k=5.1\pm0.4$ mV (similar to the values in Figure 4b). Application of 10^{-6} M BDF 9198 resulted in a slight left-ward shift in the I–V relation for I_{Peak} (closed circles): $V_{0.5}$ was −45.8±0.5 and $k=5.2\pm0.5$ mV ($P<0.001$). The I–V relation for I_{Late} (Figure 6d) shows that under control conditions there was no discernible late component of I_{Na} (open circles). In the presence of 1×10^{-6} M BDF 9198 significant I_{Late} was observable, and this showed a bell-shaped I–V relation similar in profile to, but smaller in magnitude than that for I_{Peak}. The Boltzmann fit to I_{Late} observed in the presence of BDF 9198 gave a $V_{0.5}$ of −42.5±0.7 mV and a k of 5.4±0.6 mV, parameters which were similar to those for the fit to I_{Peak} in the presence of the drug.

For a range of drug concentrations, the effect of BDF 9198 on I_{Na} (elicited by a test pulse to −30 mV) was expressed as the current density of the late current (I_{Late}), shown in Figure 6e (n=at least four cells for each concentration). There was a relatively small effect of BDF 9198 at 5×10^{-9} M, with a progressive increase in the magnitude of I_{Late} up to 1×10^{-5} M (the highest concentration applied).

Effects of BDF 9198 on the time-course of inactivation

The presence of an I_{Late} in the presence of BDF 9198 is consistent with modulation by the drug of the inactivation process, such that at the end of the 200 ms voltage command to −30 mV, substantial current still remained (Figures 5 and 6b,d). The decline of I_{Na} over a 200 ms test-pulse to −30 mV in the presence of BDF 9198 could be well described by a process comprised of two exponentials together with a residual, end-pulse component (C; see equation 2). For the cell shown in Figure 7a, the fast and slow time constants of inactivation in the presence of 1×10^{-6} M BDF 9198 were 1.50 and 55.0 ms respectively (compared to control values for the same cell of 1.48 and 12.87 ms). In a sample of six cells inactivation of I_{Na} was quantified as follows: 96±0.02% of the current was described by a τ_f of 1.4±0.3 ms; 4±0.01% of the current was fitted by a τ_s of 13.6±1.0 ms (there was no significant difference to the values obtained in Table 2, $P>0.15$). There was no discernible end-pulse current component (C). In the presence of BDF 9198, the relative proportions of the current fitted by τ_f (56±0.1%) and τ_s (16±0.2%) altered, without any significant alteration in the magnitude of τ_f (1.5±0.3 ms; $P>0.1$). By contrast τ_s was increased to 49±9.4 ms. In the presence of BDF 9198, 28±0.4% of the peak current remained at the end of the test-pulse (C). To summarize: BDF altered the relative proportions of fast to slowly inactivating I_{Na}, left the magnitude of τ_f unchanged, increased the magnitude of τ_s, and led to the presence of a significant component of I_{Na} at the end of the applied 200 ms test-pulse.

It should be noted however, that experiments with a longer duration (1 s) test-pulse indicated that the sustained current component observed 200 ms after depolarization in the presence of BDF cannot be considered to represent current amplitude in the steady-state. In 5 cells, to which 1 s pulses to −30 mV were applied 25.4±2.1% of peak inward current remained 200 ms into the pulse ($P>0.8$ compared to 200 ms pulse data above). At 1 s however, only 11.9±1.4% of peak current remained. Additionally, it was difficult to fit adequately the current decline over 1 s with a bi-exponential process. Thus, whilst inactivation of I_{Na} in the presence of BDF 9198 may adequately be ascribed a bi-exponential decline over the first 200 ms, over a longer

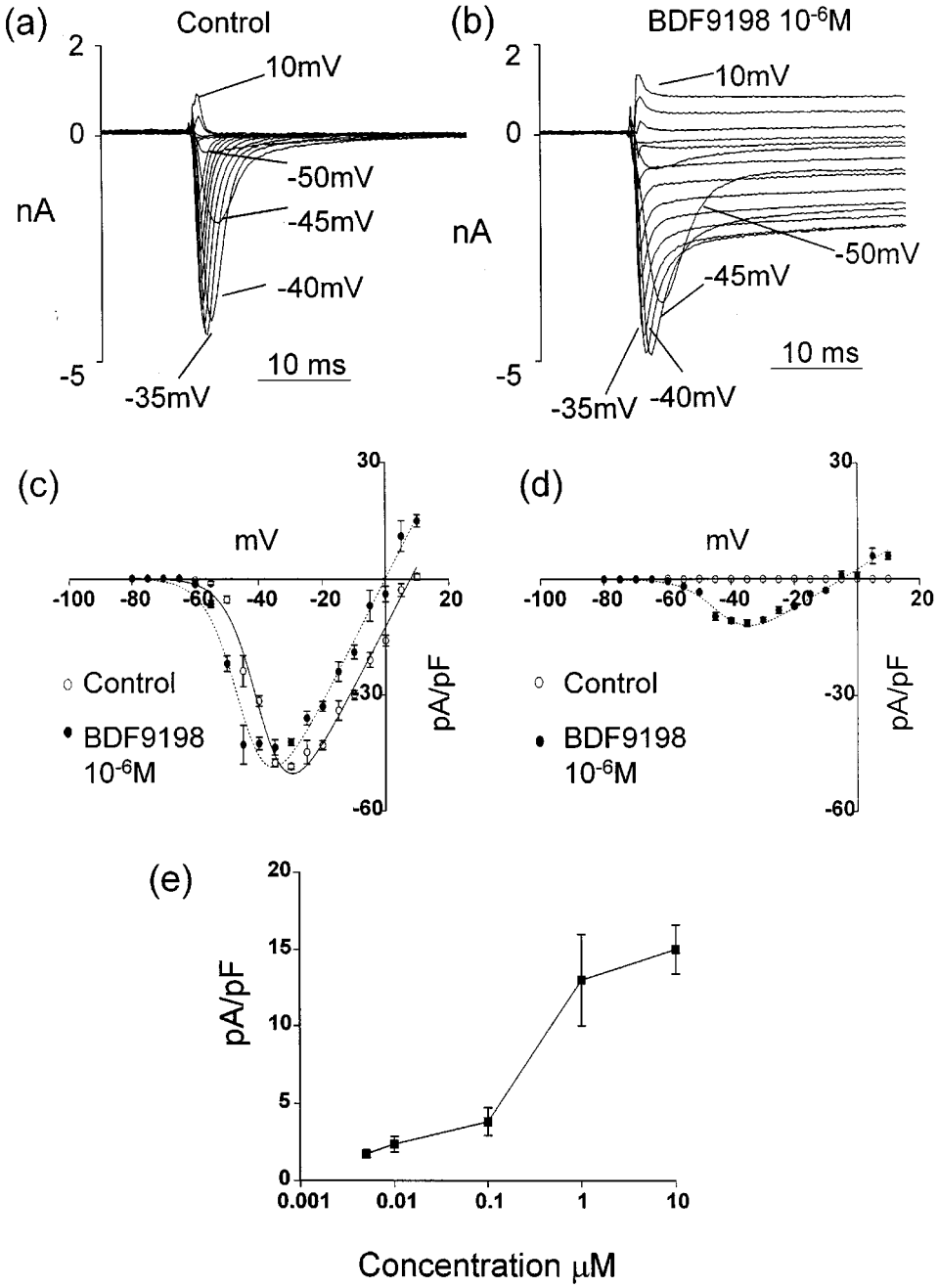

Figure 6 Voltage and concentration-dependence of effect of BDF 9198 on I_{Na}. Families of TTX-S currents elicited by step-depolarizations from −140 mV to a range of more positive test potentials under control conditions (a) and (b) in the presence of 1×10^{-6} M BDF 9198-containing solution. (c) shows the mean current–voltage (I–V) relationship for peak I_{Na} and (d) for end-pulse current (I_{Late}) under control conditions and after the application BDF 9198 1×10^{-6} M. Curve fits to the data were made using equation 1 (Results). (e) shows a concentration-response relation for the effect of BDF 9198. The magnitude of response at each concentration was expressed as the current density of drug-induced I_{Late}.

duration current inactivation may in fact be better described by process comprised of >2 exponential phases. Under our conditions it was difficult to apply long pulses in the presence of BDF 9198 and the time-course of current decline was not monitored for >1 s.

If the dominant effect of BDF 9198 is to interact with the Na channel to modulate the process of current inactivation, a sustained current component might be predicted in the presence of drug, whether I_{Na} was inwardly or outwardly directed. Figure 7b shows the result of experiments performed to test this proposition. For five cells we measured peak I_{Na} and E_{rev} and then applied a pulse to a potential an equal but opposite distance from the E_{rev}, to elicit an outward I_{Na}. Under control conditions (left panel) both inward and outward I_{Na} inactivated completely during the applied depolarization (with inactivation of the outward I_{Na} being slightly faster than that of the inward current). As expected, application of BDF 9198 led to sustained inward I_{Na} (right panel); moreover, outward I_{Na} was also modified such that a sustained current component remained at the end of the test-pulse. The results of the experiment shown in Figure 7b (displayed as TTX-S current measurements) showed that there was no significant quantitative difference between the amount of end-pulse current for inward and outward I_{Na} in the presence of BDF 9198

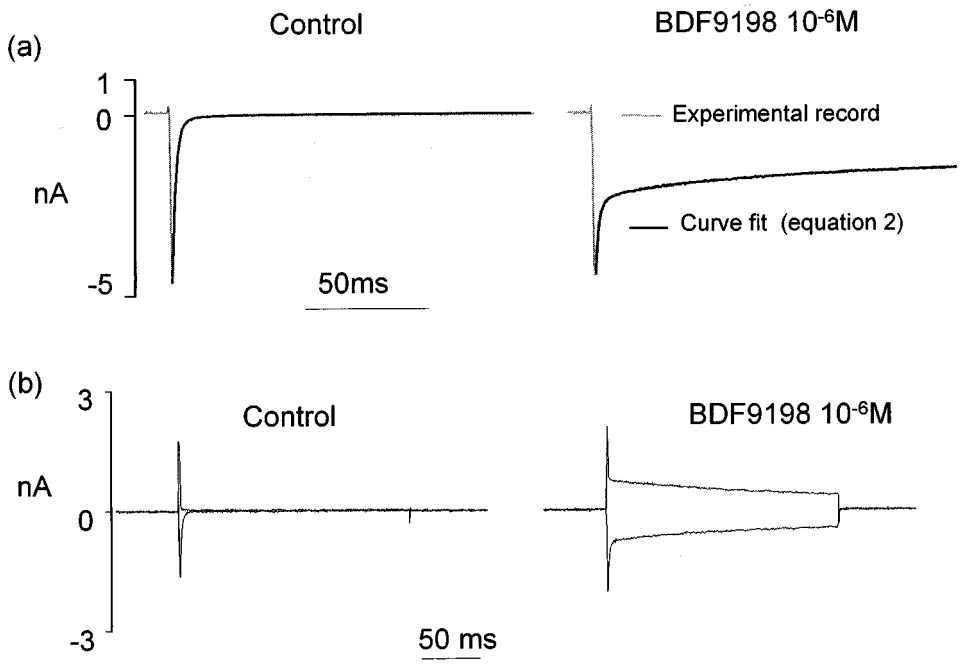

Figure 7 Effects of BDF 9198 on I_{Na} inactivation time-course. (a) shows a curve fit to the inactivation of an I_{Na} (TTX-S current) recorded under control conditions (left panel), and in the presence of 1×10^{-6} M BDF 9198 (Equation 2, see Results). Currents were elicited by a step depolarization from -140 to -30 mV. (b) shows inward and outward I_{Na} (TTX-S current) elicited in control conditions (left panel) by a step-depolarization from -140 to -30 mV and $+30$ mV (E_{rev} for $I_{Na} = 0$ mV in this cell). The effect of application of 1×10^{-6} M BDF 9198 on inward and outward I_{Na} is shown in the right panel.

(22.8 ± 2.9% and 23.6 ± 1.9% of peak current amplitude respectively, $P > 0.66$; $n =$ five cells).

Effects of BDF 9198 on I_{CaL}

The effects described for BDF 9198 on I_{Na} might be anticipated to increase the contribution of I_{Na} to membrane potential depolarization during the action potential plateau. Another important inward current that contributes to the plateau phase is I_{CaL} (e.g Linz & Meyer, 1999). Therefore, if BDF 9198 altered I_{CaL} then this could also contribute to the observed effects of the drug on APD_{50}. In order to test the effects of BDF 9198 on I_{CaL} we used selective recording conditions (see Table 1). I_{CaL} was elicited by applying a 500 ms voltage step from a holding potential of -40 to $+10$ mV. Figure 8 shows an I_{CaL} recorded under control conditions (left panel) and after the application of 1×10^{-6} M BDF 9198 (right panel). In eight cells there appeared to be no significant change ($P > 0.05$) in I_{CaL}; this precluded modulation of I_{CaL} from contributing to action potential prolongation by the drug.

Effects of BDF 9198 on I_K

We then proceeded to investigate the effects of BDF 9198 on potassium currents known to contribute to repolarization of the action potential (Carmeliet, 1993; Sanguinetti & Keating, 1997). If BDF 9198 inhibited repolarizing potassium currents, this would also contribute to action potential prolongation. We first tested the effects of BDF 9198 on the delayed rectifier current, I_K, which participates in action potential repolarization over the plateau voltage range (Sanguinetti & Keating, 1997). In guinea-pig ventricular myocytes I_K is a composite current, comprised of rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier sub-types (Sanguinetti & Jurkiewicz, 1990; Heath & Terrar, 1996). In order to record composite I_K, we applied 2000 ms duration, progressively incrementing step-depolarizations at frequency of 0.1 Hz, from a holding potential of -40 mV. Interfering currents were inhibited using the internal and external solutions described in Table 1. Figure 9a shows representative currents elicited by depolarizing pulses to four test potentials (panel b). On repolarization to -40 mV, deactivating outward tail currents were observed. Neither the currents during the test pulses, nor the outward current tails were affected by application of 1×10^{-6} M BDF 9198. Figure 9c shows mean I–V relations for the I_K tails observed on repolarization from the different test potentials ($n = 5$). There was no significant effect of BDF 9198 on I_K tail density at any test potential ($P > 0.05$), indicating that the effect of BDF 9198 on action potential duration could not be attributed to I_K inhibition.

Effects of BDF 9198 on I_{KI}

Inward rectifier potassium current (I_{KI}) participates in late repolarization of the ventricular action potential (Shimoni *et al.*, 1992; Carmeliet, 1993). We used the internal and external solutions described in Table 1 to record I_{KI} selectively. In order to elicit I_{KI} we applied a ramp protocol (Figure 10b) comprised of a step depolarization to 0 mV from a holding potential of -40 mV, followed by a descending ramp over 2000 ms to -120 mV. The protocol was applied every 3 s. In control conditions a clear inwardly rectifying current, typical of I_{KI} was observed (Figure 10a, left panel). The application of 1×10^{-6} M BDF 9198 had no significant effect on the current (right panel). This was confirmed by constructing mean I–V relations for I_{KI} (Figure 10c) in which the control data (circles) and BDF 9198 data (squares) are superimposed ($n = 6$; $P > 0.05$ at all potentials). These findings indicate that BDF 9198 did not inhibit I_{KI}, and taken together with the data in Figure 9, suggest that any action potential prolongation by BDF 9189 up to 1×10^{-6} M did not result from outward potassium current inhibition.

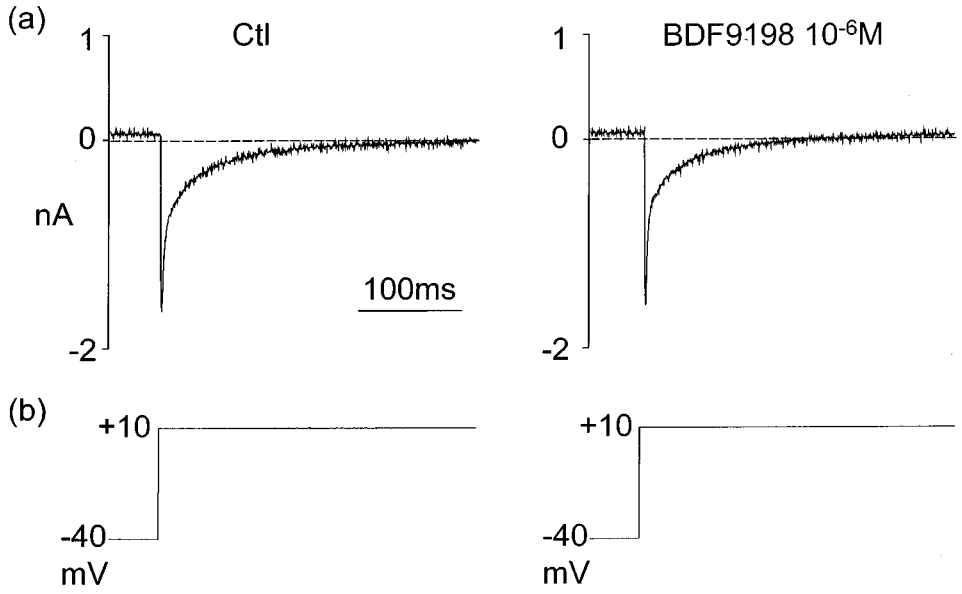

Figure 8 Effect of BDF 9198 on I_{CaL}. I_{CaL} elicited under selective recording conditions (see Methods) by applying a square voltage clamp pulse (b), from −40 to +10 mV. (a; left panel) shows current elicited in control solution (Ctl), (a; right panel) shows that 1×10^{-6} M BDF 9198 did not affect peak I_{CaL} amplitude.

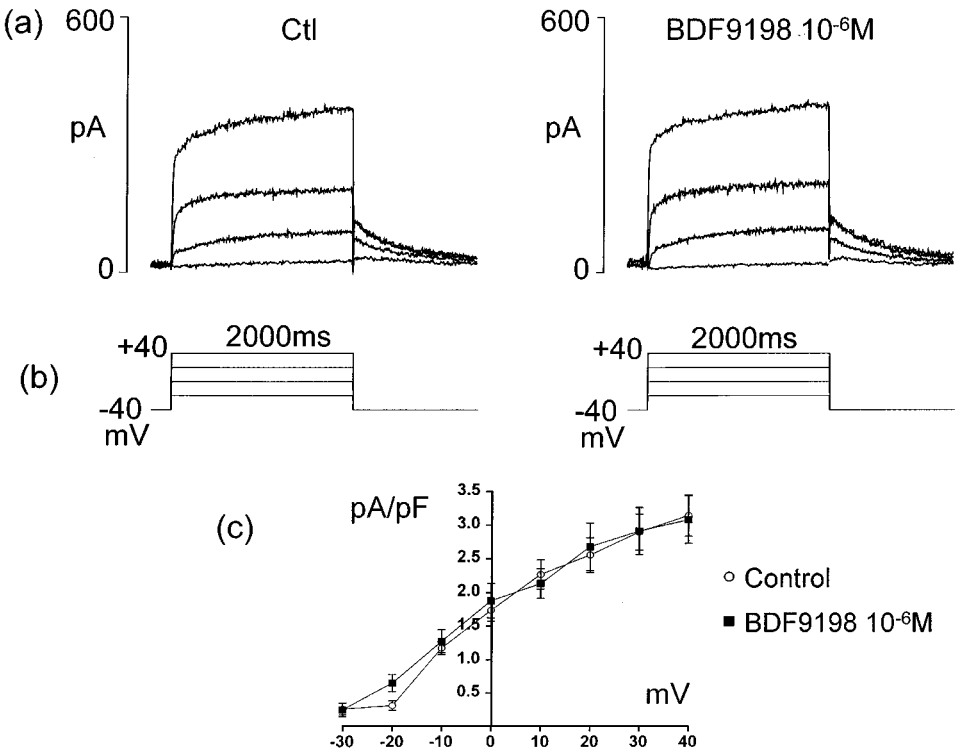

Figure 9 Effect of BDF 9198 on I_K. (a) Records of I_K (for recording conditions, see Methods) elicited by 2000 ms step-depolarizations from −40 mV to a range of test potentials (−20, 0, +20, +40 mV; b). There was no significant difference between Control (Ctl) I_K (a; left panel) and that in the presence of 1×10^{-6} M BDF 9198 (a; right panel). (c) shows the mean I-V relations for I_K tails before and after BDF 9198.

Discussion

Effects of BDF 9198 on action potentials and underlying ionic currents

BDF 9198 has recently been reported to exert a positive inotropic effect on human myocardium (Muller-Ehmsen *et al.*, 1998; Schwinger *et al.*, 1999), an effect similar to that exerted by the related compounds BDF 9148 and DPI 201-106 (e.g. Scholtysik *et al.*, 1985; Brasch & Iven, 1991; Hoey *et al.*, 1993). The present study demonstrates that BDF 9198 is also a positive inotrope on guinea-pig myocardium, showing a similar concentration-response profile to that observed with human tissue (Muller-Ehmsen *et al.*, 1998; Schwinger *et al.*, 1999). To date, however, there has been no detailed study which describes the electrophysiological effects of BDF 9198. The principle findings of the present study were that, under selective recording conditions, BDF 9198 slows inactivation of

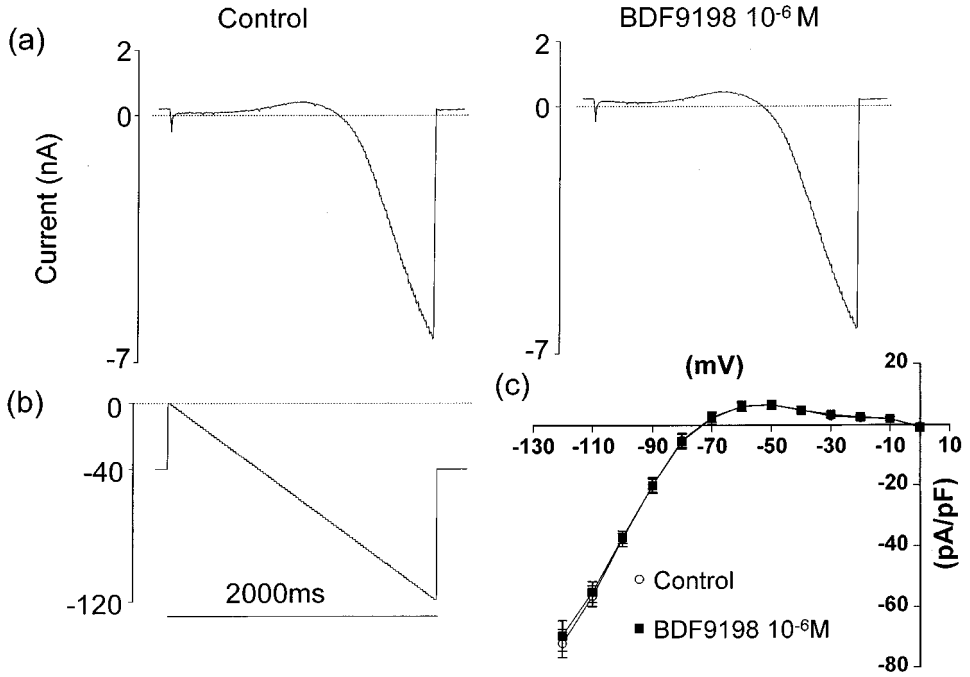

Figure 10 Effect of BDF 9198 on I_{K1}. (a) shows I_{K1} (for recording conditions, see Methods) under control conditions (left panel) elicited by a 2000 ms ramp protocol from 0 to −120 mV, applied from a holding potential of −40 mV (panel b). (Right panel) shows current elicited by the same protocol after applying 1×10^{-6} M BDF 9198. There was little observable effect of BDF 9198 1×10^{-6} M. (c) shows the mean I-V relation for I_{K1} before and after BDF 9198

I_{Na} recorded under voltage clamp, and like the related compounds BDF 9148 and DPI 201-106 (Brasch & Iven, 1991; Ravens *et al*., 1991; Hoey *et al*., 1994; Raap *et al*., 1997) the drug causes action potential prolongation. It is notable that the extent of action potential prolongation produced by BDF 9198 in the present study was markedly greater than that observed for BDF 9148 or DPI 201-106 in the guinea-pig heart. Ravens *et al*. (1991) observed a 1.2–1.3 fold increase in APD_{90} with 1×10^{-6} M BDF 9148, whilst we observed a greater than 3 fold increase in APD_{50} at a lower concentration (1×10^{-7} M). Brasch and Iven (1991) also observed comparatively modest increases in APD with BDF 9148 compared to that reported for its sister compound in the present study. Neither of these previous studies reported after-depolarizations in the presence of BDF 9148, suggesting that the degree of internal calcium loading during exposure to DPI 201-106 or BDF 9148 was smaller than that which occurred under the conditions of the present study.

The observed differences in effects on APD of BDF 9198, on the one hand, and BDF 9148 and DPI 201-106, on the other, can be explained by considering the comparative effects of the compounds on ionic currents underlying the action potential. In addition to prolonging I_{Na}, both DPI 201-106 and BDF 9148 exhibit inhibitory effects on $I_{Ca,L}$ with 30–50% inhibition at 1×10^{-6} M (Ravens *et al*., 1991). The reduction in depolarizing current resulting from this action is likely to offset, to an extent, the Na-channel effects of the compound. Under our conditions, we observed no significant alteration in peak $I_{Ca,L}$ at 1×10^{-6} M BDF 9198, thus the increased depolarizing drive of I_{Na} would not have been countered by reduced $I_{Ca,L}$. Ravens *et al*. (1991) considered the relative effects of I_{Na} prolongation and $I_{Ca,L}$ reduction in their study, noting that whilst BDF 9148 exerted more potent effects on I_{Na} than $I_{Ca,L}$, DPI 201-106 affected the currents equally, whilst producing a greater action potential prolongation. This was suggestive of additional drug effects on other ion channel types. Subsequently, differential effects of these compounds on I_{Ks}, and inward rectifier, I_{KI} were demonstrated (Amos & Ravens, 1994). DPI 201-106 produced moderate reductions in both I_{KI} and I_{Ks}, whilst BDF 9148 did not alter I_{KI} and only affected I_{Ks} at higher concentrations. In the present study, both I_{KI} and composite I_K were unaffected by 1×10^{-6} M BDF 9198–a concentration 10 fold higher than that required to prolong the action potential to over 600 ms. These observations are suggestive of selectivity of BDF 9198 for I_{Na} without concomitant changes in I_K, I_{KI} or $I_{Ca,L}$. These data suggest that the actions of BDF9198 solely on I_{Na}, underlie the action potential prolongation observed under current clamp conditions.

Effects on I_{Na}

As described in Results, the properties of I_{Na} we observed under selective conditions correlate well with other published data. The $V_{0.5}$ estimated from the fit to our control I–V data compares favourably with that reported for both feline ventricular myocytes (−33.2 mV; Shacklow *et al*., 1995) and human atrial cells (−38.9 mV, Sakakibara *et al*., 1992; −38.6 mV, Feng *et al*., 1996). Both Sakakibara *et al*. (1992) and Shacklow *et al*. (1995) found that inactivation of I_{Na} was best described by a bi-exponential decline. Our control values for τ_f and τ_s at −30 mV are close to those reported by Sakakibara *et al*, who also observed that τ_f contributed to over 90% of current inactivation at this potential.

Ravens *et al*. (1991) observed induction of a sustained I_{Na} by BDF 9148 with physiological $[Na]_o$ and showed this to be blocked by TTX. This observation is qualitatively similar to our own, regarding BDF 9198. Due to the differing $[Na]_o$, in the two studies, it is difficult to make direct comparisons between their concentration–response data for BDF 9148 and our own for BDF 9198. At 1×10^{-6} M BDF 9148 Ravens *et al*. (1991) reported a sustained I_{Na} of just under 20pA/pF in

magnitude (their Figure 4). With a 10 fold reduction in $[Na]_o$ we observed an I_{Late} of $\sim 10-12$pA/pF. This, together with the profound effects on APD_{50} does suggest that BDF 9198 may be more potent in modulating I_{Na} than its sister compound. A more recent study by Ravens and co-workers, with a lowered $[Na]_o$ (Hoey *et al*., 1994) reported no significant effects of DPI 201-106 or BDF 9148 on the I–V relation for I_{Peak}. Other data, from expressed cardiac Na channel α-subunits (Krafte *et al*., 1994), also suggested no shift in the I–V relation, but noted a small (8%) reduction in peak current with BDF 9148. The slight left-ward shift of the I–V relation for I_{Peak} we observed with BDF 9198 differs from these observations, and accounts for the larger peak currents we observed at negative potentials (e.g at −50 mV, see Figure 6b) and reduced current amplitudes at potentials on the ascending limb of the I–V curve (Figure 6b,c). Overall, we did not observe statistically significant alterations in the absolute I_{Peak} density with exposure to BDF 9198. The lack of effect of a partial blocking dose of TTX on the I–V profile for I_{Na}, despite a marked alteration in current amplitude would argue against any left-ward shift of the I–V produced by BDF 9198 resulting from an experimental artifact. However, even if present, the relatively small observed shift in voltage dependence of I_{Peak} is unlikely to have made a major contribution to the overall effect of BDF 9198 on the ventricular action potential.

The reported modification of Na currents through expressed Na channel α subunits by congeners of BDF 9198 suggests that the α subunit is the site of interaction between this family of compounds and the Na channel (Krafte *et al*, 1991; 1994). Mutagenesis experiments suggest that the III–IV linker region of the α subunit is critical for inactivation of I_{Na}, whilst TTX binds in the vestibule of the channel pore (see Fozzard & Hanck, 1996 for a review). It is possible, therefore, that BDF 9198 exerts its effects on I_{Na} inactivation by modulating the ability of the III–IV linker to act as the blocking particle normally responsible for inactivation. Such an action would produce an altered inactivation profile of the current, but would not be expected to affect the ability of TTX to inhibit current flow through BDF 9198-modulated channels. It is noteworthy that DPI 201-106 modifies inactivation time–course of current carried by cloned cardiac but not neuronal Na channel alpha-subunits expressed in *Xenopus* oocytes (Krafte *et al*., 1991). Thus, the portion of the channel to which such agents bind may differ in its molecular composition between cardiac and neuronal tissue. Expression studies, using modified α subunits are necessary to test such a hypothesis.

Implications of the study

Our data indicate that BDF 9198 is a potent modulator of cardiac I_{Na} at concentrations which do not affect other important ionic currents. As BDF 9198 is positively inotropic on the human myocardium (Muller-Ehmsen *et al*., 1998), questions therefore arise regarding the possible therapeutic value of the drug. It is difficult to predict with precision from experiments on isolated cells what effects the compound may have *in vivo*, but our results do raise issues worthy of consideration. DPI 201-106 has been reported to produce some prolongation of the QT-interval of the electrocardiogram in healthy subjects (Ruegg & Nuesch, 1995). The more extensive *in vitro* APD prolongation caused by BDF 9198 may translate into greater QT prolongation, with an associated risk of pro-arrhythmia mediated by after-depolarizations and Torsade de pointes (Roden, 1990; Levi *et al*., 1997). Such a pharmacological proarrhythmic risk may be analagous to forms of genetic Long-QT syndrome (LQTS) involving Na channel mutations which give rise to persistent I_{Na} (Bennett *et al*., 1995; Dumaine *et al*., 1996). In this regard, the selectivity of BDF 9198 for I_{Na} may make it of investigational value for experimental models of I_{Na}-linked QT prolongation. The reported effects of DPI 201-106 and BDF 9148 on additional membrane currents make them less suitable for such an application. The actual likelihood of BDF 9198-linked proarrhythmia *in vivo* would depend on whether satisfactory inotropic effects of BDF 9198 could be obtained at concentrations producing only moderate changes to the ventricular APD and, thereby, QT interval.

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