Br. J. Pharmacol. (1988), **94**, 1250–1256

Inhibitory actions of amoxapine, a tricyclic antidepressant agent, on electrophysiological properties of mammalian isolated cardiac preparations

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1 The electrophysiological effects of amoxapine were examined in guinea-pig isolated papillary muscles and rabbit sinoatrial nodes using a conventional microelectrode technique.

2 In papillary muscles, amoxapine above 10 μM caused a dose-dependent decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential and in the action potential amplitude (APA), whereas the action potential duration at 90% repolarization (APD_{90}) was significantly prolonged. For a decrease in $\dot{V}_{max}$, amoxapine produced a negative shift of the curve relating $\dot{V}_{max}$ to the resting potential (E_m) along the voltage axis to more negative membrane potentials.

3 Amoxapine also decreased $\dot{V}_{max}$ and the overshoot potential of K^+-depolarized slow action potentials of papillary muscle preparations.

4 In spontaneously beating sinoatrial node preparations, amoxapine above 3 μM reduced the heart rate, $\dot{V}_{max}$, APA and the slope of phase 4 depolarization in a dose-dependent manner.

5 It was concluded that amoxapine exerts inhibitory actions on fast- and slow- response fibres of the heart and these actions can be mainly explained by inhibition of both fast Na^+ and slow Ca^{2+} channels.

Introduction

It is well known that tricyclic antidepressants are widely used in the treatment of depressed patients and an overdose of these drugs commonly results in cardiotoxicity (Vohra *et al.*, 1975; Marshall & Forker, 1982). In electrophysiological studies, tricyclic antidepressants such as imipramine, amitriptyline, desipramine, chlorimipramine and doxepin, decreased the maximum rate of depolarization of the action potential ($\dot{V}_{max}$) in mammalian isolated fast response fibres (Tamargo & Rodriguez, 1979; Tamargo *et al.*, 1979; Brennan, 1980; Rodriguez & Tamargo, 1980; Muir *et al.*, 1982). Furthermore, imipramine depressed the slow action potentials in K^+-depolarized guinea-pig papillary muscles (Garcia de Jalon *et al.*, 1978). These findings suggest that tricyclic antidepressant drugs depress not only cardiac fast Na^+ channels but also slow Ca^{2+} channels.

Amoxapine is a tricyclic antidepressant agent of the dibenzodiazepine class, structurally related to the neuroleptic loxapine (Lydiard & Gelenberg, 1981; Smith & Ayd, 1981; Jue *et al.*, 1982). However, the effects of amoxapine on electrophysiological properties of isolated cardiac tissues have not been previously examined, except for one study using rat atria (Delgado *et al.*, 1986).

Therefore, the present study was undertaken to evaluate the electrophysiological properties of the tricyclic antidepressant compound amoxapine on guinea-pig isolated papillary muscles and rabbit sinoatrial node preparations.

Methods

Guinea-pig papillary muscles

Guinea-pigs weighing 200–300 g were stunned by a blow on the neck, the heart was quickly removed and thin (less than 1 mm) papillary muscles were excised from the right ventricle. The preparation was perfused in a recording chamber with oxygenated (95% O_2, 5% CO_2), warmed (36 ± 0.5°C) Tyrode

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solution. The composition of the normal Tyrode solution was as follows (in mM): NaCl 132.0, KCl 4.0, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.4, $CaCl_2$ 1.8 and glucose 10.0. The pH of all solutions was 7.4. The preparation was driven at 1.0 Hz for more than one hour after mounting in a chamber containing Tyrode solution. Stimulating electrodes consisted of a pair of Ag-AgCl wires (diameter 1 mm, length 5 mm) placed near the preparation. The stimulus threshold was determined during experiments before drug perfusion and the intensity of current pulses was set 1.5 times the threshold. The effects of amoxapine on the slow action potentials were examined using 0.2 mM Ba-containing Tyrode solution in which $[K^+]_0$ was increased to 27 mM.

Rabbit sinoatrial nodes

Rabbits weighing 1.5–2.0 kg were killed by a blow on the neck. The heart was quickly removed and the right atrium with sinoatrial node region was dissected in normal Tyrode solution. The sinoatrial node tissue close to the crista terminalis was cut into strands, approximately 1 mm in length and 0.2–0.3 mm in width. The pacemaking portion was ligated by two silk fibres 0.2–0.3 mm apart along the length of the strand. These small preparations of sinoatrial node were made in the same manner as in previous studies (Noma & Irisawa, 1976; Kotake *et al.*, 1985). In the present experiments, there were no changes in action potential configuration which might have suggested a pacemaker shift within the small sinoatrial node preparation. This is probably because the diameter of our preparation was less than the space constant (0.5–0.8 mm) of sinoatrial nodes studied by Bonke (1973) and Seyama (1976).

The composition of the Tyrode solution was as follows (in mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0 and NaH_2PO_4 0.6. The pH was adjusted to 7.4 by adding Na_2HPO_4. Amoxapine (Lederle Japan Ltd.) was dissolved in the distilled water, and then diluted in the Tyrode solution to the desired concentration.

Recording of the membrane potential

Transmembrane potentials were recorded with conventional glass microelectrodes filled with 3 M KCl (10–20 MΩ). The electrodes were connected to an amplifier (Nihon Kohden, MEZ 7101). Potentials were stored on a chart recorder (Nihon Kohden, RJG 4122) and displayed on an oscilloscope (Nihon Kohden VC 10).

The effect of amoxapine on the relationship between the maximum rate of rise ($\dot{V}_{max}$) and the resting potential (E_m) was studied in guinea-pig papillary muscles by changing $[K^+]_0$ from 2.7 to 5.4, 8.1, 10.8, 13.5 and 14.9 mM. After obtaining the control values, amoxapine was added to each perfusate and the effects of the different $[K^+]_0$ again examined. To make sure that $\dot{V}_{max}$ and E_m had attained a steady-state at each K^+ concentration, measurement of these parameters was made after an equilibration period of at least 7 min. Data were analysed only from the records in which a single impalement was kept throughout the experiment. The $\dot{V}_{max}$–E_m relationship was utilized as an indication of the change in steady-state Na^+ inactivation variable at different membrane potentials. We fitted the following equation proposed by Windish & Tritthart (1981) to the experimentally obtained $\dot{V}_{max}$–E_m relationship.

$$\dot{V}_{max} = \dot{V}_s/(1 + \exp[(E_m - E_f)/S])^2$$

where $\dot{V}_s$, E_f and S show the saturation value of $\dot{V}_{max}$, the membrane potential at which $\dot{V}_{max}$ is one quarter of $\dot{V}_s$, and the slope factor, respectively. These values were determined by the least squares method using a microcomputer (NEC, PC-8001). The validity of these equations for expressing the $\dot{V}_{max}$–E_m relationship in guinea-pig ventricular muscle was verified by Arita *et al.* (1983) and Hisatome *et al.* (1985).

Electrophysiological values are expressed as means ± s.d. Mean values were compared by use of Student's *t* test for paired data and *P* values less than 0.05 were considered significant.

Results

Papillary muscle preparations of guinea-pigs

The effects of amoxapine on the membrane action potential of guinea-pig papillary muscle were examined in preparations constantly driven at 1.0 Hz. Electrophysiological measurements were made 15 min after changing to a new solution. As shown in Figure 1, after exposure to amoxapine below 3 μM, no significant change in the action potential parameters was observed. Higher concentrations (above 10 μM) of amoxapine caused a decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential and the action potential amplitude (APA), and a prolongation of the action potential duration at 90% repolarization (APD_{90}) in a dose-dependent manner, whereas the resting potential was not significantly affected by the agent. The effects of amoxapine on various electrophysiological characteristics are summarized in Table 1.

In order to study the effect of amoxapine on the $\dot{V}_{max}$–E_m relationship, $[K^+]_0$ was increased from 2.7

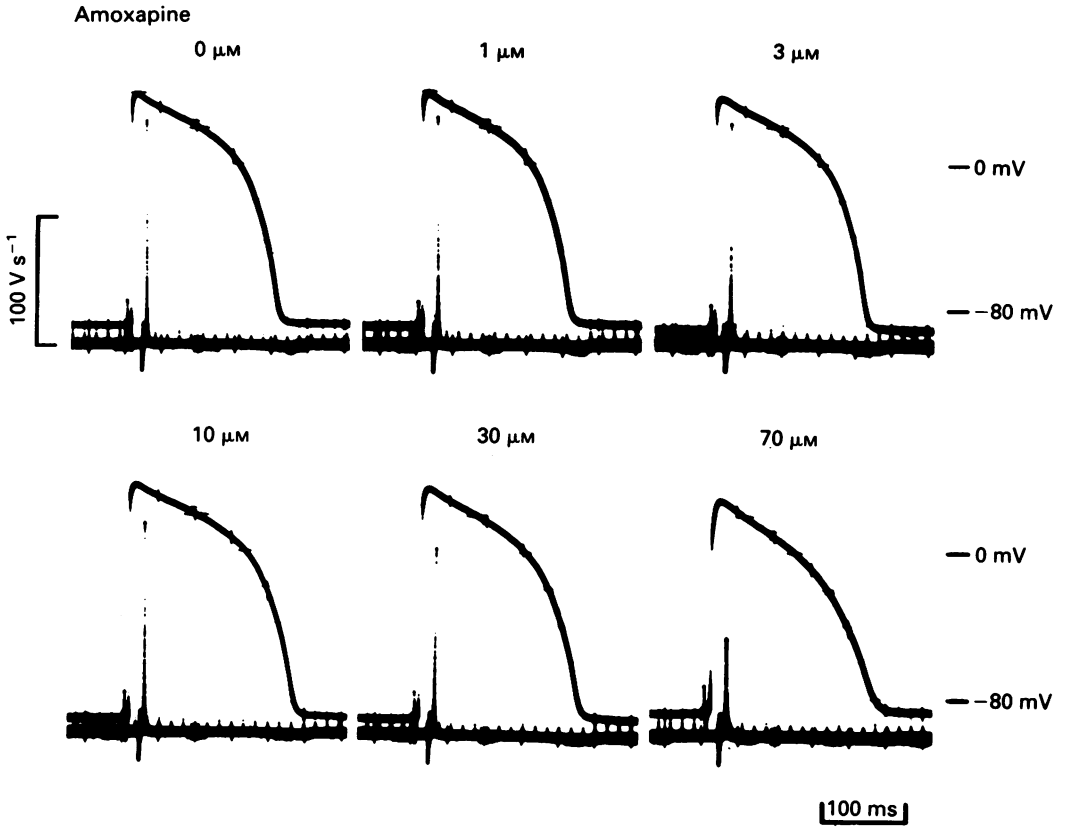

Figure 1 Effects of increasing concentrations of amoxapine on the action potential and its first derivative of guinea-pig papillary muscle.

to 5.4, 8.1, 10.8, 13.5 and 14.9 mM. $\dot{V}_{max}$ and E_m were measured during a steady-state at each K^+ concentration, and the mean value of three preparations was normalized in Figure 2. The smooth curves were drawn with the help of a computer by fitting the equation proposed by Windisch & Tritthart (1981) (see Methods). This finding indicates that the relationship of $\dot{V}_{max}$–E_m was shifted to more negative potentials after exposure to amoxapine.

The effects of amoxapine on slow action potentials

Table 1 Electrophysiological effects of amoxapine on action potentials of guinea-pig papillary muscles

	$\dot{V}_{max}$ ($V s^{-1}$)	*APA* (mV)	APD_{90} (ms)
Control	161 ± 19	137 ± 3	208 ± 6
Amoxapine (μM)			
0.1	161 ± 19	137 ± 3	208 ± 6
1	161 ± 20	137 ± 3	209 ± 6
3	160 ± 20	137 ± 2	210 ± 8
10	150 ± 19**	136 ± 3*	221 ± 6***
30	122 ± 18***	133 ± 3***	222 ± 12**
70	58 ± 21***	117 ± 6***	230 ± 14**

Values are mean ± s.d. ($n = 7$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
$\dot{V}_{max}$ = maximum upstroke velocity, APA = action potential amplitude and APD_{90} = action potential duration at 90% repolarization.

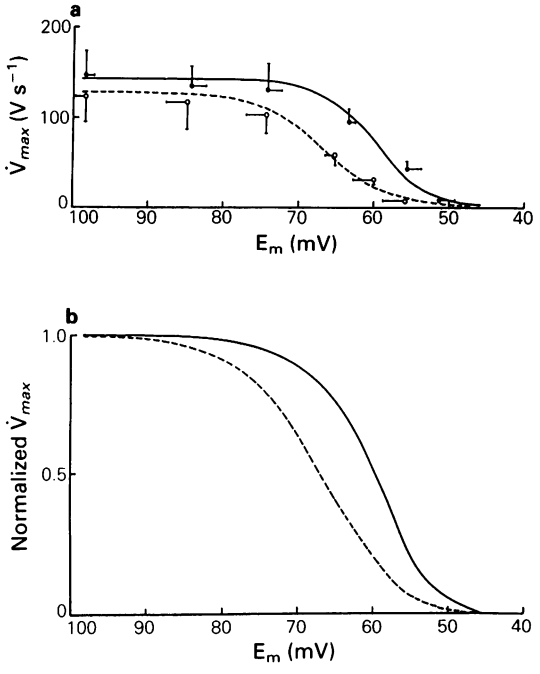

Figure 2 Effect of amoxapine (30 μM) on the relationship between the maximum upstroke velocity $\dot{V}_{max}$ and the membrane potential (E_m) of guinea-pig papillary muscle action potentials. (a) The relationship between $\dot{V}_{max}$ and E_m in the absence (●) and the presence (○) of amoxapine 30 μM ($n = 3$). (b) Normalized curve of (a) in which 1.0 on the ordinate scale corresponds to the maximum $\dot{V}_{max}$ measured in each condition. The solid line represents the control curve and the broken line represents the curve in the presence of amoxapine 30 μM.

Table 2 Electrophysiological effects of amoxapine on the slow action potentials of guinea-pig papillary muscles induced by high K^+ and 0.2 mM Ba

	$\dot{V}_{max}$ (V s^{-1})	*OSP* (mV)
Control	16.0 ± 3.0	32.8 ± 1.6
Amoxapine (μM)		
0.1	16.0 ± 3.0	32.8 ± 1.6
1	16.0 ± 3.0	32.8 ± 1.6
3	15.9 ± 3.0	32.0 ± 1.6
10	14.3 ± 2.4**	31.4 ± 1.8*
30	13.1 ± 2.2**	29.2 ± 1.6**
70	7.1 ± 1.4***	23.2 ± 3.6***

Values are mean ± s.d. ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
$\dot{V}_{max}$ = maximum upstroke velocity and OSP = overshoot potential.

evoked by increasing $[K^+]_0$ to 27 mM with 0.2 mM Ba, were examined in five guinea-pig papillary muscle preparations. As shown in Figure 3, amoxapine at above 10 μM, reduced the overshoot potential (OSP) and $\dot{V}_{max}$ of the slow action potential dose-dependently. The effects of increasing concentrations of amoxapine on various slow action potential parameters are summarized in Table 2.

Sinoatrial node preparations of rabbits

In seven spontaneously beating sinoatrial node preparations, the effect of amoxapine on the action potential was studied (Figure 4). Amoxapine above 3 μM produced a negative chronotropic effect accompanied by a decrease in $\dot{V}_{max}$, APA and the rate of diastolic depolarization (RDD). Action potential duration at 50% repolarization (APD_{50}) was significantly prolonged after exposure to 7 μM amoxapine. With 10 μM amoxapine, the spontaneous discharge disappeared in all preparations. Electrophysiological effects of amoxapine on sinoatrial node action potential characteristics are summarized in Table 3.

Discussion

Our results show that amoxapine modifies electrical activity of guinea-pig papillary muscles (above 10 μM) and rabbit sinoatrial node preparations (above 3 μM). In papillary muscles, amoxapine decreased $\dot{V}_{max}$, APA and prolonged APD_{90} in a dose-dependent manner, whereas the resting membrane potential was not affected. Furthermore, amoxapine shifted the $\dot{V}_{max}$–E_m relationship in the hyperpolarizing direction, causing a greater depression of $\dot{V}_{max}$ at more depolarized membrane potentials. From these findings, it is suggested that amoxapine depresses fast Na^+ channels, inhibits the 'residual sodium channel' (Arita *et al.*, 1983) and blocks conduction in depolarized ventricular muscle. Amoxapine also depressed slow action potentials in K^+-depolarized papillary muscle and in the sinoatrial node. In these preparations, fast Na^+ channels are almost inactivated and slow Ca^{2+} channels are mainly responsible for depolarization. Therefore, the results described above indicate that amoxapine also depresses slow Ca^{2+} channels as well as fast Na^+ channels. These electrophysiological findings are consistent with evidence that amoxapine decreased $\dot{V}_{max}$, APA, the heart rate, contractile force and prolonged APD in rat atrial fibres (Delgado *et al.*, 1986). All these effects are also similar to those previously obtained with imipramine and some neuroleptics (Langslet *et al.*, 1971; Garcia de Jalon *et al.*, 1978; Rodriguez & Tamargo, 1980; Manzanares & Tamargo, 1982).

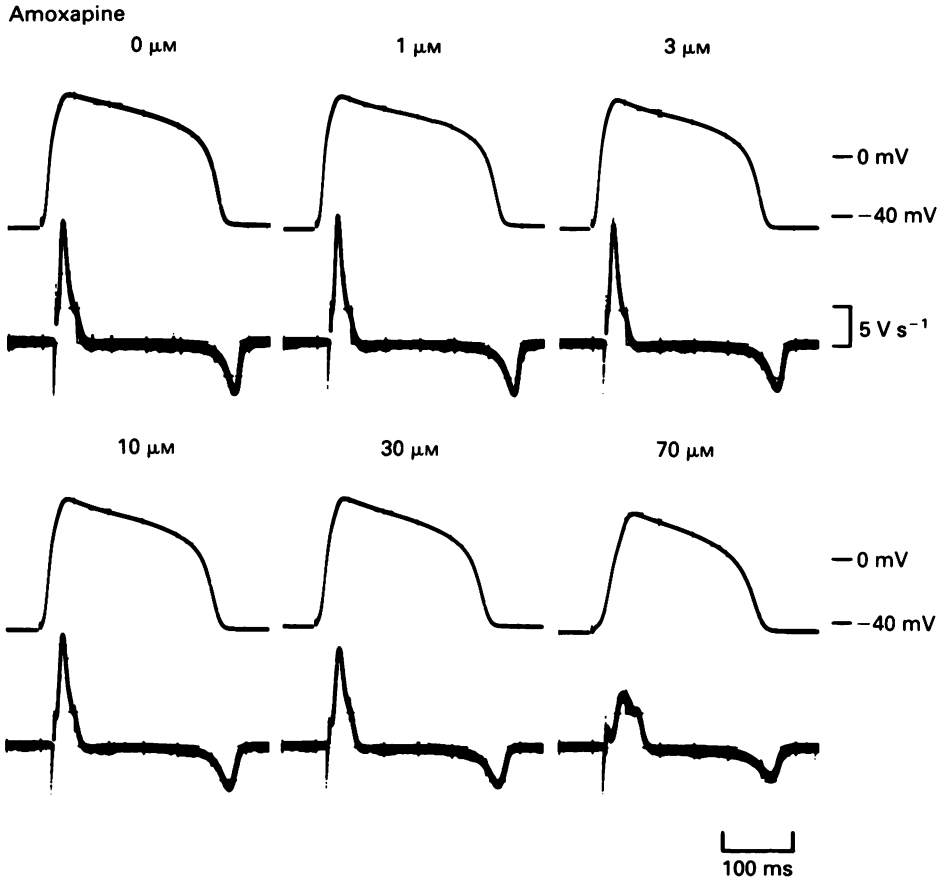

Figure 3 Effects of amoxapine on slow action potentials of guinea-pig papillary muscles induced by 27 mM K^+ and 0.2 mM Ba. The upper trace shows the transmembrane potential and the lower trace shows its first derivative.

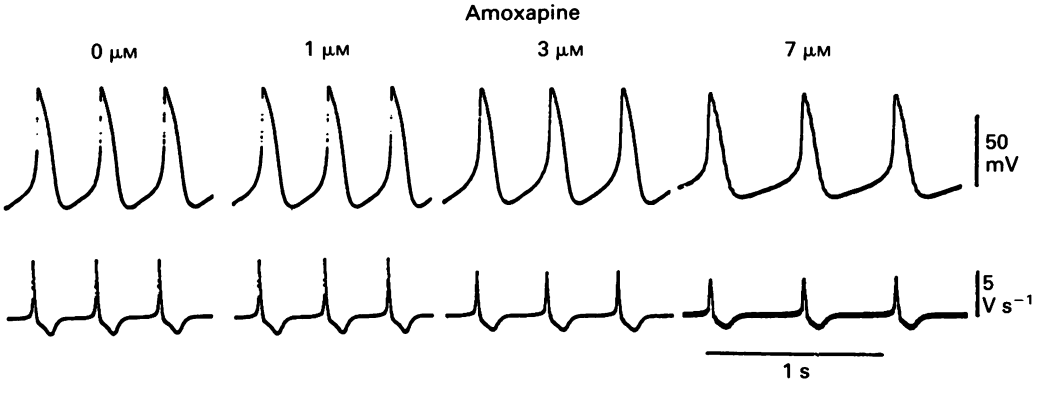

Figure 4 Effect of amoxapine on action potentials of rabbit sinoatrial node cells. The upper panel represents the action potential and the lower panel its first derivative.

Table 3 Electrophysiological effects of amoxapine on rabbit sinoatrial node action potentials

	SCL (ms)	$\dot{V}_{max}$ ($V s^{-1}$)	*APA* (mV)	APD_{50} (ms)	*RDD* ($mV s^{-1}$)
Control	330 ± 34	8.9 ± 2.4	97 ± 7	80 ± 8	109 ± 7
Amoxapine (μM)					
0.1	333 ± 35	9.0 ± 2.2	98 ± 7	79 ± 8	109 ± 4
1	349 ± 51	8.6 ± 2.3	97 ± 7	78 ± 9	106 ± 7
3	394 ± 93*	6.9 ± 1.6**	94 ± 8*	85 ± 8	94 ± 15*
7	500 ± 155**	4.1 ± 1.7***	80 ± 13**	89 ± 4**	72 ± 13**

Values are mean ± s.d. ($n = 7$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. SCL = spontaneous cycle length; RDD = rate of diastolic depolarization; for key to other abbreviations see Table 1.

In general, tricyclic antidepressant agents have been shown to cause cardiotoxic effects such as QRS prolongation, non-specific ST-T changes, atrioventricular and intraventricular block, atrial and ventricular arrhythmias, cardiac arrest and heart failure (Vohra *et al.*, 1975; Bigger *et al.*, 1978; Marshall & Forker, 1982). Amoxapine has also been shown to induce adverse cardiac effects similar to those described above (Lydiard & Gelenberg, 1981; Smith & Ayd, 1981). According to clinical studies (Boutelle, 1980; Bock *et al.*, 1982; Taylor *et al.*, 1982), therapeutic concentrations of amoxapine in man are 200–400 $ng ml^{-1}$ and concentrations after an accidental overdose are above 1000 $ng ml^{-1}$ (approximately 3 μM), respectively. In the present experiments, amoxapine 3–10 μM depressed fast Na^+ and slow Ca^{2+} channels of mammalian isolated cardiac tissues. Therefore, it is likely that such an inhibitory action can explain the cardiac toxicity observed after an overdose of tricyclic antidepressants. Although it is difficult to relate *in vivo* plasma concentrations to the concentrations of the drug perfusing isolated cardiac tissues, it is suggested that relatively high concentrations of amoxapine induce an inhibitory effect on the electrical activity of the heart, and this effect should be considered when using amoxapine as an antidepressant agent.

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(Received November 9, 1987
Accepted February 26, 1988)