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# Electrophysiological effects of amoxapine in untreated and in amoxapine-pretreated rat atria

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**1** The effects of amoxapine ($10^{-7}$–$10^{-4}$ M) have been studied in rat atrial fibres obtained from untreated animals and animals pretreated for 28 days with amoxapine (10 mg $kg^{-1}$, i.p.).

**2** In untreated atria amoxapine reduced atrial rate, contractile force and $df/dt_{max}$, prolonged the sinus node recovery time and decreased atrial excitability.

**3** Amoxapine also decreased amplitude and $V_{max}$ of the upstroke, prolonged the duration of the action potential (APD) and effective refractory period (ERP) and reduced the resting membrane potential.

**4** During the treatment with amoxapine behavioural and cardiovascular adverse effects, including hypotension, tachycardia and prolongation of the Q–Tc, were observed. However, with the exception of the ERP which was significantly prolonged in pretreated atria, pretreatment with amoxapine did not modify the control values of the measured parameters compared to those obtained in untreated atria.

**5** Further addition of amoxapine produced similar changes in both pretreated and untreated atria. However, in contrast to untreated atria, in pretreated atria the prolongation of the ERP produced by amoxapine exceeded the prolongation of the APD and thus, the ERP/APD ratio increased. The decrease in atrial excitability was also more marked in pretreated than in untreated atria.

**6** Amoxapine inhibited the slow action potentials and contractions induced by isoprenaline in K-depolarized atria.

**7** It is concluded that the electrophysiological effects of amoxapine on rat atrial fibres are similar to those described for other tricyclic antidepressants. Possible explanations for the lower cardiodepressant activity of amoxapine are discussed.

## Introduction

Amoxapine is a tricyclic antidepressant drug (TAD) of the dibenzodiazepine class, structurally related to the neuroleptic loxapine, of which it is the demethylated metabolite (Lydiard & Gelenberg, 1981; Smith & Ayd, 1981; Jue *et al.*, 1982). An overdose of a TAD commonly results in adverse cardiovascular effects including hypotension, supraventricular and ventricular arrhythmias, conduction block and congestive heart failure (Vohra *et al.*, 1975; Marshall & Forker, 1982). Amoxapine overdose, in contrast, has not been associated with severe cardiotoxicity (Goldberg & Spector, 1982; Jue *et al.*, 1982) even when sinus tachycardia, prolongation of the QRS complex and non-specific ST-T changes have been reported during amoxapine therapy (Lydiard & Gelenberg, 1981; Smith & Ayd, 1981). However, recently Bock *et al.* (1982) described one patient who developed sinus tachycardia, an incomplete right bundle branch block and non-specific S-T segment and T wave change during treatment with amoxapine. Moreover, amoxapine-induced atrial flutter, which returned within 24 h to sinus rhythm after withdrawal of the drug, has been reported (Zavodnick, 1981). All these results suggest that amoxapine might exert cardiac toxicity such as that described for other TADs.

The cardiac electrophysiological effects of classical tricyclic antidepressants – imipramine (Rawling & Fozzard, 1979; Rodriguez & Tamargo, 1980) desipramine (Tamargo *et al.*, 1979), chlorimipramine (Tamargo & Rodriguez, 1979) and doxepin (Brennan, 1980) – have been attributed to the inhibition of the fast inward Na current. Moreover, very recently Manzanares & Tamargo (1982) have demonstrated that the inhibition of the fast inward Na current induced by imipramine was potentiated in rats chronically treated with imipramine. Unfortunately, the

electrophysiological effects of amoxapine have not been previously studied in isolated cardiac fibres. Therefore, the present study was undertaken to investigate (a) the electrophysiological effects of amoxapine in rat isolated atria and (b) whether these effects were affected by pretreatment of the animals for 28 days with amoxapine.

## Methods

Sprague-Dawley rats (200–300 g) were stunned by a blow on the head and their hearts rapidly removed. Right and left atria were excised and mounted in tissue baths containing Tyrode solution equilibrated with 95% $O_2$–5% $CO_2$ and maintained at 34°C. The Tyrode solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $NaHCO_3$ 11.9 and glucose 5.5. Left atria were stimulated at a basal rate of 3 Hz with square-wave pulses (1 ms duration, twice threshold strength) delivered by a pair of platinum wire electrodes. Patterns of basic and premature stimuli were controlled by a multipurpose programmable stimulator. The atria were preloaded with 1 g and the force and rate of contractions were recorded isometrically on a Grass polygraph using a Grass FT03 force-displacement transducer. All preparations were allowed to equilibrate for at least 30 min before control measurements were made.

Transmembrane action potentials (APs) were recorded through glass microelectrodes filled with 3 M KCl (tip resistance 15–30 MΩ). The microelectrodes were connected via Ag-AgCl half cells to high impedance capacity neutralizing amplifiers. The APs were displayed on a storage oscilloscope (Tektronix 5104N) and photographed on film with a Grass camera as described elsewhere (Tamargo *et al.*, 1979; Rodriguez & Tamargo, 1980). The following parameters were measured from APs recorded from atrial fibres: resting potential, amplitude, maximum rate of depolarization ($V_{max}$), action potential duration (APD) at 50% and 90% of repolarization and the effective refractory period (ERP). $V_{max}$ was obtained by electronic differentiation. The ERP was measured by delivering premature test stimuli of twice threshold strength at different intervals from the preceding driving stimulus; interpolation and shift along time axis were carried automatically every eighth basic drive stimuli (Tamargo *et al.*, 1979). The strength-duration curves were calculated from the relationship between the duration of the cathodal pulse (0.5–30 ms) and the minimum current required to evoke a contraction. Stimulus amplitude was monitored on an oscilloscope as the voltage drop across a 20 MΩ resistor in series with the stimulating electrodes. To measure the sinus node recovery time, right atria were driven at 5 Hz for 30 s. The stimulation was then stopped and the period of asystole between the last driven beat and the first spontaneous beat was measured. In another set of experiments, following equilibration atria were rendered inexcitable by depolarizing with 27 mM $K^+$ Tyrode solution. Excitability, i.e. slow APs and contractions, were restored in depolarized atria stimulated at a basal rate of 0.12 Hz by adding isoprenaline ($10^{-6}$ M) to the perfusate. In order to compare the results, the control values of the measured parameter in each experiment were taken as 100% and compared to those obtained 30 min after each increment in drug concentration.

### *Experiments in amoxapine-treated atria*

In another group of experiments rats were injected with amoxapine (10 mg $kg^{-1}$ daily i.p.) or propylene glycol (1 mg $kg^{-1}$) for 28 days. To determine the effectiveness of the regime adopted for the administration of the drugs, changes in systolic blood pressure, heart rate and electrocardiograms (ECG) were monitored during the treatment in conscious rats. Lead II ECGs were recorded with subcutaneous needle electrodes on a Grass polygraph and heart rate was measured directly from a ratemeter. Systolic blood pressure was measured by the tail-cuff method in rats prewarmed in thermostatic cages to 37°C. Recordings were made before and 24 h after the previous dose of amoxapine on days 2, 7, 14, 21 and 28 of treatment. R–R, P–R, QRS and Q–T intervals were measured and Q–Tc derived from $Q\text{–}Tc = QT/\sqrt{R\text{–}R}$. The animals were killed 24 h after the final injection and both right and left atria were set up for measurement of the recorded contractile and electrophysiological parameters as described above. Pretreament with propylene glycol for 28 days did not modify the AP characteristics compared to control values obtained in untreated animals (Table 1), thus these data were omitted in the results.

### *Drugs*

Drugs used were: amoxapine hydrochloride (generously supplied by Lederle) and isoprenaline hydrochloride (Sigma). Amoxapine was prepared as a concentrated stock solution ($10^{-2}$ M) in a 20% solution of propylene glycol. Further dilutions were carried out in Tyrode solution to obtain the final desired concentrations between $10^{-7}$ M and $10^{-4}$ M, equivalent to 0.031–31.3 μg $ml^{-1}$. In order to test the effect of the solvent, an equivalent concentration of propylene glycol was added alone in some experiments; no obvious effects were observed. Ascorbic acid ($10^{-4}$ M) was added to prevent oxidation of isoprenaline. All concentrations refer to those of the salt.

**Table 1** Electrophysiological effects of amoxapine in untreated rat atrial fibres

| *Drug concentration* (M) | *Resting potential* (mV) | *Amplitude* (mV) | $V_{max}$ ($V s^{-1}$) | $APD_{50}$ (ms) | $APD_{90}$ (ms) | *ERP* (ms) |
|---|---|---|---|---|---|---|
| 0 (13) | 87.2 ± 0.7 | 105.6 ± 1.3; | 107.4 ± 4.8 | 12.1 ± 1.1 | 36.4 ± 4.2 | 32.5 ± 2.0 |
| $10^{-7}$ (13) | 87.5 ± 1.0 | 105.5 ± 1.5 | 104.4 ± 4.3 | 15.5 ± 1.7* | 46.4 ± 3.8* | 37.0 ± 1.7* |
| $10^{-6}$ (13) | 86.3 ± 1.0 | 103.8 ± 1.5 | 88.5 ± 4.5** | 16.2 ± 1.6** | 52.3 ± 4.6*** | 40.2 ± 2.4** |
| $10^{-5}$ (13) | 82.2 ± 2.2* | 97.8 ± 3.3* | 80.0 ± 4.1*** | 16.7 ± 1.4*** | 59.8 ± 4.3*** | 51.6 ± 3.7*** |
| $5 \times 10^{-5}$ | 71.3 ± 2.4*** | 79.5 ± 4.5*** | 34.5 ± 6.5*** | 21.3 ± 2.2*** | 68.7 ± 7.4*** | 71.0 ± 5.9*** |
| $10^{-4}$ | 64.5 ± 2.2*** | — | — | — | — | — |

Values are mean ± s.e.mean. Number of observations ($n$) in parentheses.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Throughout the paper results are expressed as mean ± s.e.mean. Statistical significance was determined by Student's $t$ test and differences were considered significant when $P<0.05$.

## Results

### *Effect of chronic treatment with amoxapine*

Two groups of 8 rats were treated with amoxapine for 28 days as described. Following treatment with the drug both behavioural and cardiovascular changes appeared. Behavioural changes included sedation, depression of locomotor activity, slight to marked ataxia and catalepsy. ECG and systolic blood pressure were monitored before and on the 2nd, 7th, 14th, 21st and 28th day after the start of the treatment. After 2, 7 and 14 days of treatment there were no significant changes in R–R, P–Q and QRS intervals, whereas the Q–Tc was significantly prolonged on the 7th ($P<0.05$) and 14th ($P<0.001$) day, but not on the 2nd day. After 21 and 28 days of treatment a significant ($P<0.05$) prolongation of the R–R interval accompanied the prolongation of the Q–Tc ($P<0.001$) but no significant changes in P–R interval and QRS width were observed. A significant decrease in systolic blood pressure was also observed during the treatment with amoxapine. This hypotensive effect became statistically significant on the 2nd day of treatment (97.5 ± 1.2 mmHg as compared to 120.4 ± 3.2 mmHg, $P<0.01$) and after 28 days of treatment systolic blood pressure remained significantly reduced compared to values obtained before the treatment began (107.5 ± 3.5 mmHg compared to 120.4 ± 3.2 mmHg ; $P<0.05$).

### *Effect on spontaneously beating right atria*

Figure 1a shows concentration-response curves for the effects of amoxapine ($10^{-7}-5 \times 10^{-5}$ M) on atrial rate and contractile force in control, untreated atria. In 28 spontaneously beating right atria the control values for both parameters were 243.5 ± 17.5 beats $min^{-1}$ and 486.7 ± 76.0 mg, respectively. Amoxapine at concentrations $\geq 10^{-7}$ M produced a significant decrease in atrial rate which at concentrations higher than $10^{-6}$ M was accompanied by a significant negative inotropic effect. Thus, at $10^{-5}$ M amoxapine suppressed the spontaneous activity in 3 atria, in 11 at $2.5 \times 10^{-5}$ M and in the remaining 14 at $5 \times 10^{-5}$ M. In 17 pretreated right atria, control values for atrial rate (257.8 ± 12.5 beats $min^{-1}$) and contractile force (404.6 ± 67.5 mg) were similar to those obtained in untreated atria ($P>0.05$). As is shown in Figure 1b amoxapine, $10^{-7}-5 \times 10^{-5}$ M, produced dose-dependent negative chronotropic and inotropic effects which paralleled the effects observed in untreated atria. Spontaneous activity was suppressed at $10^{-5}$ M in 2 atria, in 7 at $2.5 \times 10^{-5}$ M and in the remaining 8 at $5 \times 10^{-5}$ M.

Amoxapine not only produced a negative chronotropic effect but also prolonged the sinus node recovery time (SNRT). In 10 untreated right atria the SNRT averaged 244.5 ± 11.2 ms and in the presence of amoxapine $10^{-7}$, $10^{-6}$ M, significantly increased by 14.8 ± 2.8% ($P<0.01$), 35.8 ± 5.7% ($P<0.01$) and 118.8 ± 19.6% ($P<0.001$), respectively. In another 10 pretreated right atria the control value for the SNRT was not significantly different from that obtained in untreated atria (281.0 ± 19.3 ms; $P>0.05$). Amoxapine ($10^{-7}-10^{-5}$ M) prolonged the SNRT by 10.9 ± 5.4% ($P<0.05$), 19.8 ± 9.8% ($P<0.05$) and 47.0 ± 10.8% ($P<0.01$), respectively. These results indicate that the prolongation of the SNRT was significantly higher at $10^{-6}$ M ($P<0.05$) and $10^{-5}$ M ($P<0.01$) in untreated than in pretreated atria.

### *Effect on electrically driven left atria*

In 10 left atria stimulated at a basal rate of 3 Hz isometric parameters of contractile force were: peak

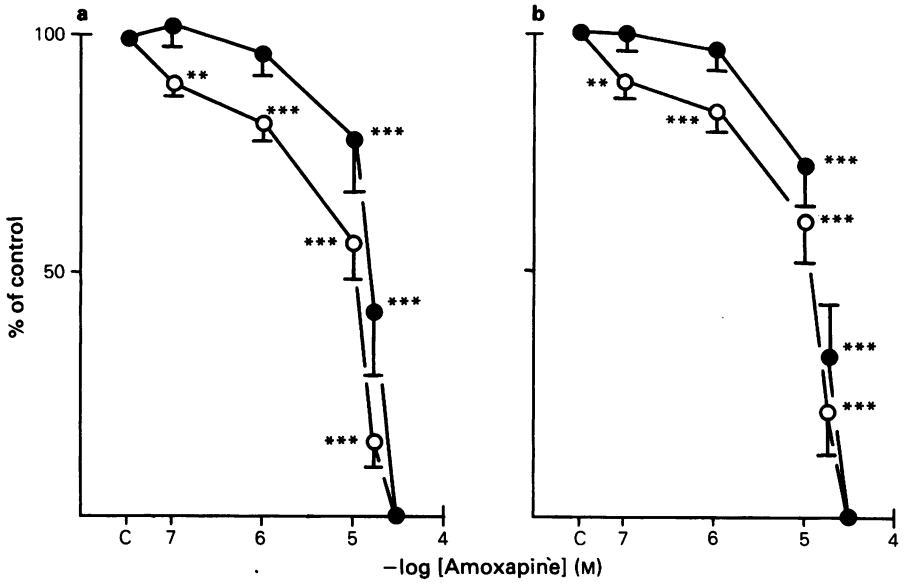


**Figure 1** Effect of amoxapine on atrial rate (○) and peak contractile force (●) in spontaneously beating right atria obtained from untreated (a) and from amoxapine-treated (b) rats. Each point represents the mean, with vertical lines showing s.e.mean, of at least 10 experiments. $*P<0.05$; $**P<0.01$; $***P<0.001$.

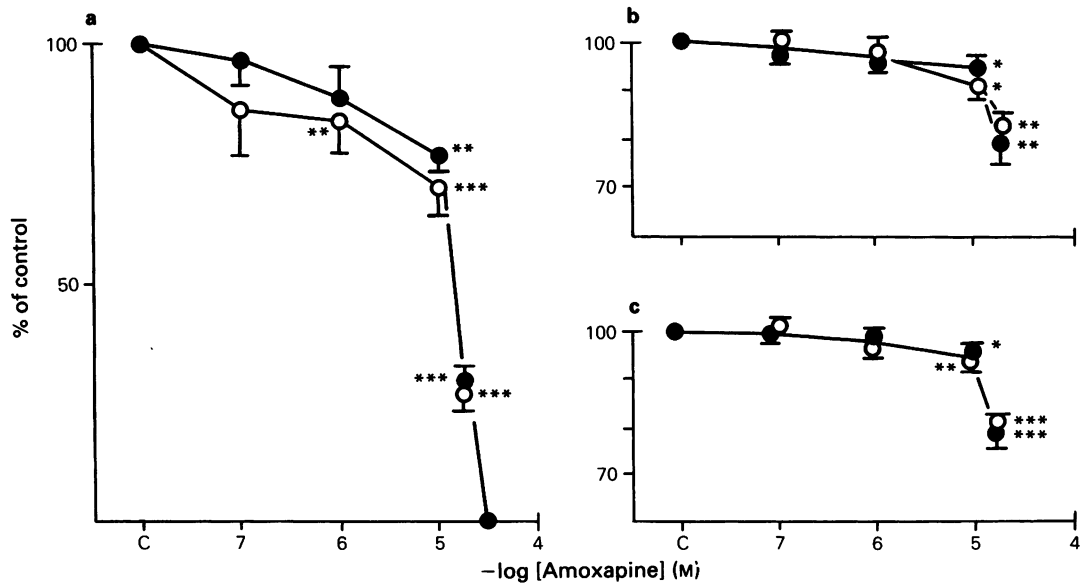


**Figure 2** Effect of amoxapine on contractile force (a), time to peak tension (b) and time for total contraction (c) in electrically driven left atria obtained from untreated (○) and amoxapine-treated (●) rats. Each point represents the mean, with vertical lines showing s.e.mean, of at least 10 experiments. $*P<0.05$; $**P<0.01$; $***P<0.001$.

contractile force = 581.7 ± 80.3 mg, $df/dt_{max}$ = 14.6 ± 2.9 mg ms$^{-1}$, time to peak tension = 39.6 ± 1.3 ms and time for total contraction = 133.5 ± 3.2 ms. Amoxapine ($10^{-7}$–$5 \times 10^{-5}$ M) produced a dose-dependent parallel decrease in contractile force (Figure 2) and $df/dt_{max}$ (not shown) which reached significant values at concentrations $> 10^{-6}$ M. At concentrations between $10^{-5}$ M and $2.5 \times 10^{-5}$ M the negative inotropic effect of amoxapine was accompanied by a significant shortening of the time to peak tension and time for total contraction. Contractile force was reduced to almost zero and all fibres became inexcitable 5–10 min after amoxapine $5 \times 10^{-5}$ M, was added to the bath. Resting tension was not significantly affected by amoxapine at any concentration tested. Since contractile force depends on the rate of stimulation, the negative inotropic effect of amoxapine was studied in 8 left atria driven at frequencies between 0.5 and 5 Hz (Figure 3). Under control conditions peak contractile force increased by 219.3 ± 23.3% when the stimulation rate decreased from 5 to 0.5 Hz. Amoxapine, $10^{-7}$ M and $10^{-6}$ M, reduced contractile force by a similar amount at all stimulation rates and when the rate of stimulation decreased from 5 to 0.5 Hz the increase in contractile force averaged 219.4 ± 16.4% and 216.9 ± 25.8%

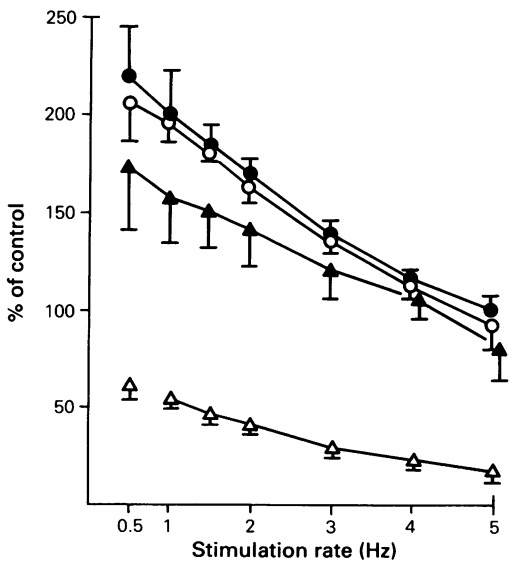


**Figure 3** Effect of amoxapine on the force-frequency relationship in electrically driven left atria. Ordinate scale: % of control values (values obtained at 5 Hz were taken as 100%). Control (●). Amoxapine, (○) $10^{-7}$ M, (▲) $10^{-6}$ M and (△) $2.5 \times 10^{-5}$ M. Each point represents the mean, with vertical lines showing s.e.mean, of 9 experiments.

($P > 0.05$). However, at $2.5 \times 10^{-5}$ M, the reduction of contractile force was more pronounced at fast than at slow frequencies and when the rate of stimulation decreased from 5 to 0.5 Hz the peak contractile force increased by 315.0 ± 28.6% ($P < 0.01$).

In another 10 pretreated left atria, control values for peak contractile force (540.6 ± 49.2 mg), $df/dt_{max}$ (13.0 ± 1.1 mg ms$^{-1}$), time to peak tension (40.7 ± 1.1 ms) and time for total contraction (130.5 ± 2.6 ms) were similar to those obtained in untreated atria ($P > 0.05$). Amoxapine $> 10^{-5}$ M, exerted a negative inotropic effect which was accompanied by a reduction in the $df/dt_{max}$, time to peak tension and time for total contraction similar to those described in untreated atria (Figure 2).

*Electrophysiological effects on amoxapine*

The effects of amoxapine ($10^{-7}$–$10^{-4}$ M) on AP characteristics were studied in 13 untreated left atria driven at a basal rate of 3 Hz. Results are summarized in Table 1. Atria were exposed to each concentration of amoxapine for 30 min, the period of time required to produce stable changes in AP characteristics. Amoxapine, $10^{-7}$ M, did not modify the amplitude and $V_{max}$ of the AP or the resting membrane potential. At $10^{-6}$ M, amoxapine significantly reduced the $V_{max}$, whereas at higher concentrations it produced a significant reducion of the amplitude and $V_{max}$ of the AP which was accompanied by a progressive shift of the resting membrane potential to less negative values. At $10^{-4}$ M, the resting membrane potential was depolarized to − 64.5 ± 2.2 mV and all fibres became inexcitable within 10 min. Moreover, at any concentration tested, amoxapine produced a dose-dependent slowing in the speed of repolarization during phase 2 and 3 which led to a lengthening of the APD at both 50% and 90% of repolarization. Amoxapine also caused a significant prolongation of the atrial ERP which paralleled the lengthening of the APD. Thus, the ERP/APD ratio remained unchanged even after exposure to $10^{-5}$ M amoxapine (0.95 ± 0.07 compared to 0.91 ± 0.08; $P > 0.05$). The effects produced by concentrations of amoxapine $> 10^{-5}$ M were only partly reversed during washout with control Tyrode solution.

The electrophysiological effects of amoxapine were also studied in 13 left atria obtained from animals pretreated with amoxapine for 28 days and driven at a basal rate of 3 Hz. As is shown in Table 2, the control values for AP characteristics in amoxapine pretreated atria were similar to those obtained in untreated atria, except for the ERP which was significantly prolonged ($P < 0.01$) compared to values obtained in untreated atria (Table 1). Moreover, the effects of increasing concentrations of amoxapine were almost similar in both groups of experiments and within 5–10 min of

**Table 2** Electrophysiological effects of amoxapine in rat atrial fibres pretreated for 24 days with amoxapine (10 mg kg$^{-1}$ daily i.p.)

| *Drug concentration* (M) | *Resting potential* (mV) | *Amplitude* (mV) | $V_{max}$ (V s$^{-1}$) | *$APD_{50}$* (ms) | *$APD_{90}$* (ms) | *ERP* (ms) |
|---|---|---|---|---|---|---|
| 0 (13) | 85.8 ± 1.6 | 106.8 ± 2.0 | 108.0 ± 3.8 | 12.1 ± 1.9 | 32.8 ± 4.4 | 45.7 ± 2.9 |
| $10^{-7}$ (13) | 83.4 ± 0.8 | 106.1 ± 1.7 | 102.0 ± 3.0 | 19.9 ± 2.7* | 51.9 ± 5.1* | 50.8 ± 3.1 |
| $10^{-6}$ (13) | 82.6 ± 0.5 | 106.7 ± 1.5 | 88.9 ± 4.6** | 17.9 ± 2.4* | 51.7 ± 5.1* | 60.4 ± 5.5* |
| $10^{-5}$ (13) | 82.1 ± 0.6* | 104.2 ± 1.4 | 84.5 ± 4.2*** | 23.1 ± 3.2** | 67.7 ± 5.4*** | 80.2 ± 7.5*** |
| $5 \times 10^{-5}$ (11) | 75.2 ± 1.6*** | 79.8 ± 4.3*** | 34.5 ± 4.7*** | 29.4 ± 4.4*** | 79.3 ± 8.8*** | 274.8 ± 54.9*** |
| $10^{-4}$ (11) | 64.9 ± 3.0*** | — | — | — | — | — |

Values are mean ± s.e.mean. Numbers in parentheses indicate the number of experiments.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

perfusion with $10^{-4}$ M all fibres depolarized to − 64.1 ± 1.3 mV and became inexcitable. The only significant difference between untreated and pretreated atria was in the prolongation of the ERP produced as the concentration of amoxapine in the perfusate increased. The ERP was significantly lengthened by increasing the concentration of amoxapine and at concentrations $> 10^{-6}$ M this prolongation was significantly higher ($P<0.01$) in pretreated than in untreated atria. Moreover, in pretreated atria the lengthening of the ERP produced by amoxapine exceeded the prolongation of the $APD_{90}$. Thus, in contrast to that in untreated atria, in pretreated atria the ERP/$APD_{90}$ ratio was significantly prolonged in atrial fibres exposed to $5 \times 10^{-5}$ M amoxapine (2.50 ± 0.48 compared to 0.99 ± 0.07; $P<0.01$).

*Effect on atrial excitability*

The effect of amoxapine on atrial excitability was studied in left atria in which strength-duration curves were obtained. At $10^{-6}$ M, amoxapine did not modify atrial excitability in either untreated (Figure 4) or pretreated atria (not shown). However, at $2.5 \times 10^{-5}$ M, amoxapine significantly increased ($P<0.05$) the stimulus current required at each stimulus duration and shifted the strength-duration curves upward and to the right increasing the rheobase. This effect was more evident in amoxapine-pretreated than in untreated atria (Figure 4). In other words, amoxapine decreased atrial excitability.

*Effect of amoxapine on slow action potentials and contractions*

The effects of amoxapine on the slow Ca channels were studied in partially depolarized atrial fibres. Following the equilibration period atria were perfused with Tyrode solution containing 27 mM $K^+$ and stimulated at 0.12 Hz. Under these conditions the resting membrane potential was shifted to − 44.3 ± 0.8 mV ($n = 7$. Table 3), the fast Na channels were inactivated and atrial fibres did not follow electrical stimulation unless isoprenaline ($10^{-6}$ M) was added to the perfusate. Amoxapine at concentrations between $10^{-7}$ M and $10^{-6}$ M did not modify the amplitude of the slow contractions or the characteristics of the slow APs induced by isoprenaline. At higher concentrations

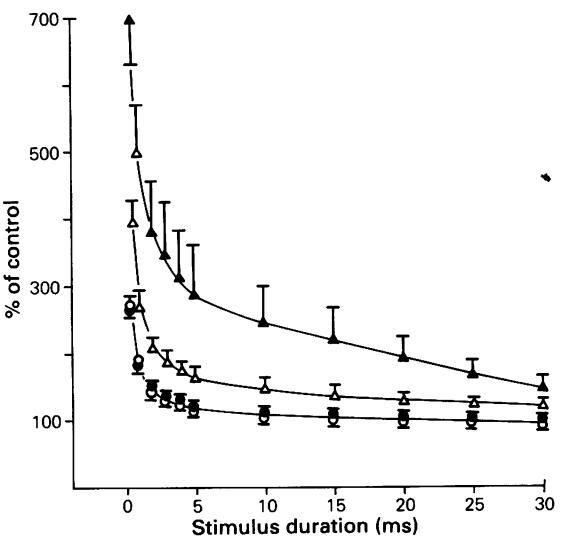


**Figure 4** Effect of amoxapine on the strength-duration curves in electrically driven left atria. Ordinate scale: minimum current required to evoke a contractile response (% of control values). Abscissa scale: duration of the stimuli (ms) at that time. Results were obtained under control conditions (●) and after exposure of untreated atria to amoxapine, $10^{-6}$ M (○) and $2.5 \times 10^{-5}$ M (△), and amoxapine-pretreated atria to amoxapine $2.5 \times 10^{-5}$ M (▲). Each point represents the mean of 7 experiments. Vertical lines indicate s.e.mean.

**Table 3** Electrophysiological effects of amoxapine on the slow action potentials induced by isoprenaline in K-depolarized rat atrial fibres

| *Drug concentration* (M) | *Resting potential* (mV) | *Amplitude* (mV) | $V_{max}$ ($V s^{-1}$) | $APD_{50}$ (ms) | $APD_{90}$ (ms) |
|---|---|---|---|---|---|
| 0 | 44.3 ± 0.8 | 67.9 ± 2.1 | 8.7 ± 1.0 | 64.5 ± 3.5 | 88.3 ± 5.3 |
| $10^{-5}$ | 43.8 ± 0.9 | 65.0 ± 2.7 | 7.5 ± 1.0 | 75.5 ± 7.9 | 98.8 ± 9.5 |
| $2.5 \times 10^{-5}$ | 44.3 ± 1.1 | 58.8 ± 1.6** | 5.3 ± 0.8** | 76.5 ± 8.9 | 106.6 ± 8.8 |
| $5 \times 10^{-5}$ | 42.6 ± 1.0 | 36.0 ± 3.9*** | 2.3 ± 0.5*** | 46.2 ± 4.9** | 88.8 ± 9.8 |
| $10^{-4}$ | 40.8 ± 0.9* | | | | |

Values are mean ± s.e.mean; $n = 7$.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

($10^{-5}$, $2.5 \times 10^{-5}$ and $5 \times 10^{-5}$ M) amoxapine decreased dose-dependently the amplitude of the slow contractions by 13.5 ± 3.0% ($P < 0.05$), 36.5 ± 6.7% ($P < 0.01$) and 68.0 ± 10.6% ($P < 0.001$), respectively. Between $10^{-5}$ M and $2.5 \pm 10^{-5}$ M amoxapine also produced a dose-dependent decrease in the amplitude and $V_{max}$ of the slow APs (Table 3) without altering the APD or the resting membrane potential. At $5 \times 10^{-5}$ M, these effects were accompanied by a shortening of the $APD_{50}$. Within 5 min of perfusion with $10^{-4}$ M, amoxapine suppressed the slow APs and contractions and the resting membrane potential was arrested at − 40.8 ± 0.09 mV. The inhibitory effect of amoxapine was partially reversed by increasing the $[Ca^{2+}]_o$ from 1.8 mM (control) to 3 mM.

## Discussion

In this paper the effects of amoxapine were studied on rat isolated atrial fibes obtained from untreated and amoxapine-treated animals. The wide range of concentrations tested (31.4–31340 ng $ml^{-1}$ or $10^{-7}$–$10^{-4}$ M) covered both therapeutic concentrations (200–500 μg $ml^{-1}$; Boutelle, 1980) and those found after an accidental overdose in man (>1000ng-$ml^{-1}$ or $> 3 \times 10^{-6}$ M; Bock *et al.*, 1980; Taylor *et al.*, 1982). Moreover, pretreatment of the rats with amoxapine produced adverse behavioural and cardiovascular effects similar to those previously described in rats and in patients given amoxapine (Greenblatt *et al.*, 1978; Chermat *et al.*, 1979; Lydiard & Gelenberg, 1981; Jue *et al.*, 1982). These results clearly indicated that in our experiments the animals were treated with an effective dosage regime.

In the present experiments amoxapine: (a) reduced atrial rate, peak contractile force and $df/dt_{max}$; (b) decreased amplitude and $V_{max}$ of the fast APs which suggest that it inhibits the fast inward Na current responsible for depolarization; (c) depolarized the resting membrane potential which explains a further reduction in phase 0 characteristics of the AP, and decreased atrial excitability; (d) prolonged the SNRT and the duration of the AP and ERP, and (e) decreased the amplitude and $V_{max}$ and shortened the APD of the slow APs. All these effects are similar to those previously described with imipramine (Matsuo, 1967; Garcí de Jalón *et al.*, 1978; Rodriguez & Tamargo, 1980; Manzanares & Tamargo, 1982) and with some neuroleptics (Langslet *et al.*, 1971; Arita & Surawicz, 1973; Stimmel, 1979).

At concentrations lower than those which affected phase 0 characteristics amoxapine prolonged the $APD_{50}$ and $APD_{90}$ values. This prolongation of the APD could be attributed to an increase in the slow inward Ca current, $I_{Ca}$, and/or to a decrease in the outward K current (Carmeliet & Vereecke, 1979). The results of this paper show that amoxapine decreased peak contractile force and inhibited the slow APs and contractions induced by isoprenaline in atrial fibres where the fast Na channels were voltage-inactivated following perfusion with 27 mM $K^+$ Tyrode solution. However, these effects were observed only at concentrations of amoxapine $> 10^{-5}$ M (3140 μg $ml^{-1}$), that is, at concentrations far above those observed even after an accidental overdose. Therefore, the possible inhibitory effect of amoxapine on $I_{Ca}$ could be of little, if any, relevance to clinical use of the drug. Thus, the prolongation of the ADP is most probably explained by a decrease in $G_K$. In fact, imipramine reduced $^{42}K$ efflux in the perfused rat heart (Langslet *et al.*, 1971) and the outward K current in isolated ventricular myocytes (Isenberg & Tamargo, 1985). This prolongation was accompanied by a parallel prolongation of the ERP, so that the ERP/APD ratio remained unaltered. These results are in contrast to those demonstrating that at therapeutic concentrations imipramine inhibited the slow APs (García de Jalón *et al.*, 1978), reduced the $I_{Ca}$ (Isenberg & Tamargo, 1985) and prolonged the ERP/APD ratio (Rodriguez & Tamargo, 1980). These differences could explain why at therapeutic concentrations amoxapine does not

exhibit the proarrhythmic/antiarrhythmic effects described for imipramine (Bigger *et al.*, 1978; Marshall & Forker, 1982).

A major finding of this study is that after effective chronic treatment with amoxapine control values of the measured parameters were similar, except those of ERP, in untreated and in pretreated atria. However, following the addition of amoxapine to the perfusate two differences between pretreated and untreated atria became evident. First, the decrease in atrial excitability was more marked in pretreated than in untreated atria. Second, the prolongation of the ERP was significantly higher in pretreated atria than that in untreated atria and exceeded the prolongation of the APD and, therefore, the ERP/APD ratio increased. However, these two differential effects were observed only with concentrations of amoxapine above $10^{-5}$ M. Thus, the addition of therapeutic concentrations of amoxapine to the perfusate produced similar changes in untreated and pretreated atria. These results are in contrast to those obtained with imipramine (Manzanares & Tamargo, 1982) and could explain why adverse cardiovascular effects are rarely observed during chronic treatment of depressed patients with amoxapine (Smith & Ayd, 1981; Jue *et al.*, 1982). One possible reason for the differences between imipramine and amoxapine could be related to the pharmacokinetics of these compounds. Both drugs are metabolized to active metabolites with a prolonged serum half-life, desipramine and 8-hydroxyamoxapine, respectively. However, the cardiodepressant effects of desipramine are even more marked than those of imipramine (Tarmago *et al.*, 1979), whereas in animal studies (Lydiard & Gelenberg, 1981; Jue *et al.*, 1982) and clinical trials (Boutelle, 1980; Jue *et al.*, 1982) the cardiovascular effects of 8-hydroxyamoxapine are similar to those of the parent compound. Thus, the prolonged effects observed in this paper when amoxapine (which had a half-life of about 8 h in the rat) was chronically administered once daily can be attributed, at least in part, to its metabolite 8-hydroxyamoxapine.

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