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Evidence that oxmetidine inhibits transmembrane calcium flux in cardiac and vascular tissue

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1 Oxmetidine, at concentrations in excess of 1×10^{-6}M, caused concentration-dependent negative inotropic and chronotropic responses in guinea-pig isolated heart preparations.

2 Oxmetidine, at concentrations in excess of 1×10^{-5}M, caused negative inotropic responses in guinea-pig papillary muscle preparations. The negative inotropic responses to oxmetidine were associated with shortening of the plateau phase of the action potential.

3 Verapamil and nifedipine caused similar shortening of the plateau phase of the action potential at equivalent negative inotropic concentrations indicating that oxmetidine may also act as a calcium antagonist.

4 In preparations partially depolarized by raising extracellular K^+ concentration, oxmetidine also exhibited negative inotropic activity and reduced the calcium-dependent action potential. However, unlike verapamil and nifedipine, oxmetidine did not show voltage-dependent activity.

5 Oxmetidine, at concentrations in excess of 1×10^{-5}M, inhibited Ca^{2+}-dependent contractions of dog saphenous vein preparations and inhibited $^{45}Ca^{2+}$-uptake into veins depolarized by high extracellular K^+.

6 *In vivo*, these calcium antagonist actions of oxmetidine were demonstrated by vasodilatation, reduction in blood pressure, bradycardia and reduced cardiac output in anaesthetized cats.

7 Oxmetidine, at concentrations of 1×10^{-5}M and above, shows properties consistent with inhibition of transmembrane Ca^{2+} flux. This action can be distinguished from other calcium antagonists as the effects of oxmetidine are not voltage-dependent.

Introduction

Oxmetidine is a potent histamine H_2-receptor antagonist (Blakemore, *et al.*, 1980), which inhibits the actions of histamine at concentrations of 1×10^{-7}M and above. Coruzzi *et al.* (1983) have recently shown that oxmetidine, at concentrations in excess of 1×10^{-5}M, elicits negative chronotropic and inotropic activity on isolated cardiac preparations from guinea-pigs, rabbits and man. These actions of oxmetidine could be reversed by increasing concentrations of extracellular Ca^{2+} or isoprenaline, suggesting that oxmetidine may interfere with transmembrane calcium flux.

We have now investigated further the actions of oxmetidine on the heart and on blood vessels *in vitro* and *in vivo* and made further analysis of the effect of oxmetidine on Ca^{2+} flux in these tissues. Actions of oxmetidine have been compared with the actions of verapamil and, to a lesser extent, with those of nifedipine.

Methods

Guinea-pig isolated working heart preparation

The isolated working guinea-pig heart preparation has been described in detail elsewhere (Flynn *et al.*, 1979). In brief, perfusion medium (modified Krebs-Henseleit bicarbonate buffer) equilibrated with 5% CO_2 in O_2 at 37.5°C, entered the left atrium at a fixed filling pressure of 10 cmH_2O. Ventricular contraction ejected fluid through the aorta against a column of height 70 cm. For the assessment of cardiac performance, the following parameters were measured: $dLVP/dt_{max}$

(ventricular contractility), sinus rate, aortic flow, coronary flow, cardiac output and stroke volume.

The exposure of hearts to oxmetidine was achieved by the addition of suitable concentrations to the circulating perfusion medium. An ascending concentration scheme was employed, the hearts being allowed to achieve a stable response before each increase in concentration. All experiments were completed within a period of approximately 1 hour, over which hearts are very stable (Flynn *et al.*, 1978). Changes in parameters were expressed as absolute changes from control values taken before exposure to a drug.

Guinea-pig cardiac electrophysiology

Guinea-pigs weighing approximately 500 g were killed by cervical dislocation followed by exsanguination, the hearts rapidly removed and placed in Krebs-Henseleit buffer (37°C; equilibrated with 5% CO_2 in O_2) to allow expulsion of residual blood. The heart was then transferred to a dissection dish containing perfusion medium, the right ventricle opened and a suitable papillary muscle selected and carefully removed. A loop of cotton was attached to the papillary muscle close to the ventricle wall and a length of cotton to the opposite end. The preparation was then removed from the heart and mounted horizontally between two silver stimulating electrodes, in the tissue chamber (vol. 4 ml) of a plexiglass organ bath. The length of cotton was attached to an isometric tension transducer (Havard 0363-060) and the preparation stretched to the apex of the pre-load active tension curve, and allowed to stabilise for approximately 1 hour. The tissue chamber was maintained at 37°C (± 0.1°C) and perfused with Krebs-Henseleit buffer (pre-equilibrated with 5% CO_2 in O_2), at a flow rate of 6.00 $cm^3 min^{-1}$ (Pharmacia peristaltic pump P1). Preparations were electrically stimulated to contract at 0.2 Hz with electrical square wave pulses of 1 ms duration, at threshold + 10% intensity, from a Grass S11 stimulator. The organ bath and associated apparatus was mounted on a vibration isolation table (Ealing Beck Ltd), inside a Faraday cage, to minimize vibrational and electrical interference. Intracellular potentials were determined using classical electrophysiological techniques. In brief, glass micro-electrodes (tip diameter $< 1.0\,\mu m$; end tip resistance in 3 M KCl 5–20 MΩ) were mounted on a silver/silver chloride recording electrode connected to a voltage follower (WPI 701) possessing input capacity neutralisation. The tip of the micro-electrode was positioned into individual myocardial cells using a Prior micromanipulator and the signal obtained displayed on a Nicolet Explorer I digital storage oscilloscope, together with the force of contraction. The upstroke velocity of the action potential (dV/dt) was assessed from the first differential of the output from the voltage follower, using an operational amplifier (Hugo Sachs Ltd). The signals obtained from the force transducer, voltage follower and operational amplifier were recorded on magnetic tape using a Racal 4DS Fm tape recorder (band width 0–10 kHz) and were subsequently replayed for quantitative analysis. Hard copies of the data were obtained via the use of an X-Y plotter (Allen Datagraph model 815) and a polaroid camera (Shackman 7000 using polaroid black and white film).

Following the period of stabilisation, impalements were attempted. Successful impalements were allowed to mature and then left for 15 min to ensure that the action potential was stable.

The effects of drugs were investigated by their addition in solution to the perfusion medium. Experiments with nifedipine were carried out in the absence of light.

Studies on force of contraction and action potential configuration in partially depolarized preparations

Preparations were set up as described above. Following stabilisation of preparations, the K^+ content of the Krebs Henseleit solution was increased from 5.9 mM to 27 mM with an equivalent reduction in the Na^+ content. This procedure results in a partial depolarization of the resting membrane potential and is known to cause the virtual abolition of the fast sodium inward current responsible for the upstroke of the normal action potential Weidmann (1955). The action potential under these conditions is largely due to the slow, Ca^{2+}-dependent inward current. Under these conditions, larger stimulation voltages were needed to elicit slow response action potentials.

Studies in dog saphenous vein preparations

Inhibition of KCl-induced calcium contraction Mongrel dogs of either sex were killed by an overdose of sodium pentobarbitone. The lateral saphenous vein was removed and cleared of connective tissue. Segments of saphenous vein, 2–4 mm, were prepared and suspended, under 2 g applied tension, in 10 ml organ baths maintained at 37°C in a physiological salt solution of composition (mM): NaCl 138, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgCl_2.6H_2O$ 1.2, HEPES 5.0 and glucose 11.1 bubbled with 100% O_2. Propranolol, 1×10^{-6}M and cocaine 3×10^{-5}M, were added to this solution. Tissues were initially washed 3–4 times followed by a 60 min equilibration period. The muscles were then contracted by 3 consecutive additions of 80 mM KCl, each addition being followed by a 15 min wash and re-equilibration period. The external Ca^{2+} was then removed by 2 washes, each 1 min in duration, using the Ca^{2+}-free physiological salt solution to which 2 mM EGTA had been added. These washes

were followed by 2 further washes in a physiological salt solution free of $CaCl_2$ and EGTA.

The physiological salt solution additionally contained SK&F 101253 (9-allyloxy-6-chloro-3-methyl-2,3,4,5-tetrahydro-1H-benzazepine) an α_1-adrenoceptor antagonist (De Marinis *et al.*, 1984), at 1×10^{-4}M to eliminate any muscle contraction which might occur due to release of catecholamines when the preparations were depolarized by addition of KCl.

Following stabilisation of the tissue, KCl, (80 mM) was added to the organ bath; 5 min later, a $CaCl_2$ concentration-response curve was constructed over the concentration range 1×10^{-6} to 1×10^{-1}M. The preparation was then washed to remove the $CaCl_2$. Antagonists were equilibrated with the tissue for 30 min. The Ca^{2+} concentration-response curve in the K^+-depolarizing medium was then determined.

$^{45}Ca^{2+}$-uptake studies $^{45}Ca^{2+}$-uptake was measured in dog saphenous vein preparations under conditions described by Karaki & Weiss (1979) and Jim *et al.* (1984). Tissue rings of 3–5 mm width were equilibrated at 37°C for 1 h in a physiological salt solution of the following composition (mM): NaCl 140, KCl 5, $CaCl_s.2H_2O$ 2.5, $MgCl_2.6H_2O$ 1, glucose 10 and HEPES 5, pH 7.3–7.4, amd constantly bubbled with pure O_2. Propranolol, 1×10^{-6}M, and cocaine, 3×10^{-5}M were added to this solution. The tissues were then incubated in this physiological salt solution labelled with $^{45}Ca^{2+}$ (0.5 μCi ml^{-1}) for 30 min, which was the time for maximal uptake under non-stimulated (basal) conditions, followed by incubation in the $^{45}Ca^{2+}$-labelled physiological salt solution containing equimolar NaCl substituted KCl (80 mM, 30 min). After this procedure, the tissues were put into an ice-cold Ca^{2+}-free La^{3+} solution (75.1 mM $LaCl_3$, 10 mM glucose and 5 mM HEPES) for 1 h. The tissues were then blotted on Whatman No. 1 filter paper and weighed. The $^{45}Ca^{2+}$ taken up by the tissues was then extracted by overnight incubation in 2.5 ml EDTA (5 ml mM) at room temperature (Meisheri *et al.*, 1981). Finally 7.5 ml of scintillation solution containing Triton X-100 was added and vials analysed for $^{45}Ca^{2+}$ in a Searle (Mark III) liquid scintillation counter. The tissue $^{45}Ca^{2+}$-uptake was calculated from the formula,

$$^{45}Ca^{2+}\text{-uptake} = \frac{\text{d.p.m. in muscle}}{\text{wet wt (kg)}} \times \frac{\text{mmol Ca l}^{-1}}{\text{d.p.m. l}^{-1}}$$

When studying the effect of drugs on KCl-stimulated $^{45}Ca^{2+}$-uptake, the tissues were exposed to a $^{45}Ca^{2+}$-labelled physiological salt solution containing various concentrations of drug for 30 min before incubation in the same solution containing KCl (80 mM). The effect of drugs on basal $^{45}Ca^{2+}$-uptake was studied by incubating the tissue rings in a $^{45}Ca^{2+}$-labelled physiological salt solution for 1 h in the absence and presence of various concentrations of drug.

Haemodynamic studies in anaesthetized cats

Experiments have been done in 4 cats, body weight 2.45 kg, anaesthetized by an intraperitoneal injection of sodium pentobarbitone 60 mg kg^{-1}. The trachea was cannulated. Blood pressure was measured from a cannula tied into the right femoral artery. Oxmetidine was administered via a cannula tied into the right brachial vein.

With the cat maintained by positive artificial respiration, the thorax was opened and the ascending aorta dissected to allow placement of an electromagnetic flow probe around the vessel. The size of the probe was selected to provide a tight fit around the aorta.

Blood pressure, heart rate (monitored from the blood pressure pulse) and cardiac output were recorded directly and total peripheral resistance and stroke volume calculated from these directly measured parameters.

Oxmetidine was administered by continuous intravenous infusion at an infusion rate of 0.16 ml min^{-1}. Infusions were made at 8×10^{-8}, 4×10^{-7}, 2×10^{-6} and 1×10^{-5} mol kg^{-1} min^{-1} and were each of 10 min duration with an interval of 15 min between infusions. This dosing schedule was identified in the first of the cats and the results from the subsequent three animals in which this procedure was followed have been used.

Drugs used

Oxmetidine dihydrochloride (SK&F); verapamil (a gift from Knoll A.G. Ludwigshafen, FRG); $^{45}CaCl_2$ (34.28 mCi mg^{-1}) was purchased from NEN, Boston, Mass.; $LaCl_3.7H_2O$ from Aldrich, Milwaukee, U.S.A.; Triton X-100 from Rohm & Haas, Philadelphia, U.S.A.; cocaine HCl from Merck, Sharpe & Dohme, Rahway, U.S.A.; nifedipine (a gift from Bayer A.G. Frankfurt, FRG), SKF 101253 (synthesized by Dr DeMarinis, SKF Laboratories, Philadelphia, U.S.A.).

Results

Guinea-pig isolated working heart preparation

Oxmetidine caused concentration-dependent depression of cardiac function. The threshold concentration to elicit bradycardia, a reduction in dp/dt_{max}, aortic flow, cardiac output and stroke volume was approximately 1×10^{-6}M and concentration-dependent de-

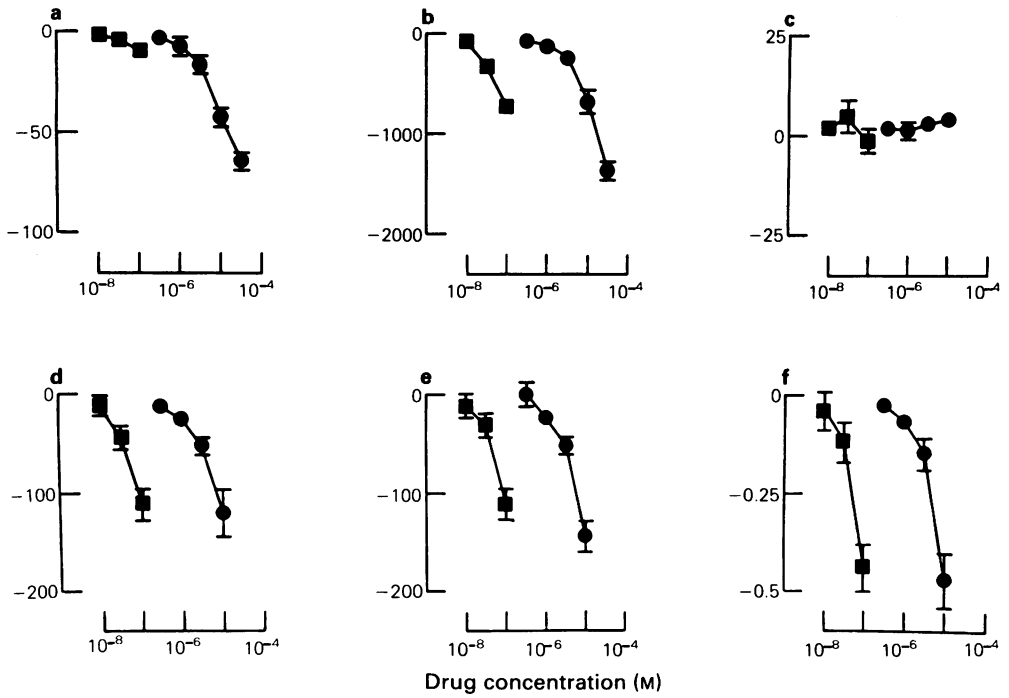

Figure 1 Guinea-pig isolated working heart. Changes in (a) heart rate, beats min^{-1}; (b) dp/dt_{max}, mmHg s^{-1}; (c) coronary flow, ml min^{-1} g^{-1} dry weight; (d) aortic flow, ml min^{-1} g^{-1} dry weight; (e) cardiac output, ml min^{-1} g^{-1} dry weight and (f) stroke volume, ml, in hearts exposed to oxmetidine (●) or verapamil (■). Values are mean with s.e.mean shown by vertical lines; $n = 5$.

pression occurred until preparations failed to pump against the fixed afterload at 3.16×10^{-5}M (Figure 1). Oxmetidine had little effect on coronary flow.

Verapamil caused concentration-dependent reductions in dp/dt_{max}, aortic flow, cardiac output and stroke volume over the concentration range 1×10^{-8} to 1×10^{-7}M, above which all hearts failed (Figure 1). Verapamil had minimal effects on heart rate or coronary flow over the dose-range studied.

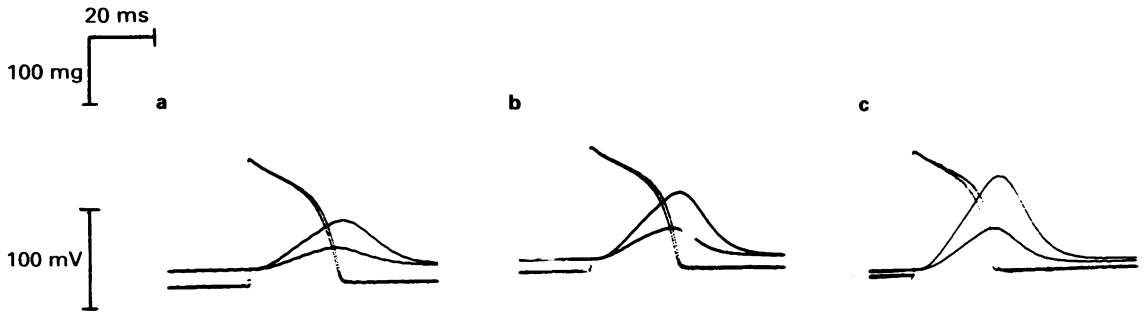

Figure 2 Guinea-pig papillary muscle, contraction and action potential configuration. Treatment with (a) oxmetidine 1×10^{-4}M; (b) verapamil, 1×10^{-5}M; or (c) nifedipine, 3.16×10^{-7}M, reduces the force of contraction by about 50% and shortens the plateau phase of the action potential. In each record the upper contraction curve is the control response and the lower curve that in the presence of drug. The outer action potential is the control and the inner action potential that in the presence of drug. In all cases, drug exposure was for 15 min.

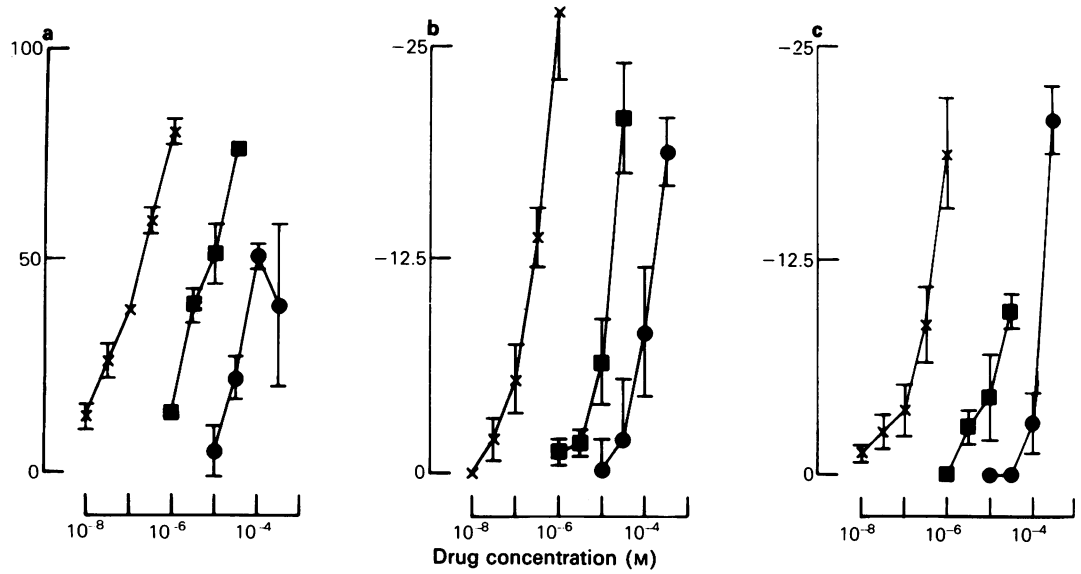

Figure 3 Guinea-pig papillary muscle, contraction and action potential configuration. The actions of nifedipine (X), verapamil (■) and oxmetidine (●) in reducing (a) the force of muscle contraction, (% inhibition), (b) shortening the action potential duration to 20% repolarization, (ms), and (c) the action potential duration to 90% repolarization, (ms). Points are mean with s.e.mean shown by vertical lines; $n = 3$ for nifedipine and verapamil, $n = 5$ for oxmetidine.

Guinea-pig cardiac electrophysiology

Oxmetidine reduced the force of papillary muscle contraction and caused a reduction in the plateau phase of the action potential (action potential duration to 20% repolarization, APD_{20}) at 3×10^{-5}M and 1×10^{-4}M without causing changes in resting potential, action potential magnitude (APM), maximum rate of rise of the action potential or action potential duration to 90% repolarization (APD_{90}) (typical experimental record, Figure 2). At 3×10^{-4}M, the force of contraction of the preparations increased, variably and transiently, the plateau phase of the action potential was further shortened despite the partial reversal of the negative inotropic action of oxmetidine. At this higher concentration, APD_{90} was also significantly shortened whereas the other variables of the action potential remained unaltered (Figure 3).

Verapamil reduced the force of papillary muscle contraction over the dose range, 1×10^{-6} to 3×10^{-5}M. Like oxmetidine, verapamil reduced the plateau phase of the action potential (Figures 2 and 3) and at the highest concentration also reduced APD_{90}. At these concentrations, verapamil did not significantly change resting membrane potential, APM or the maximum rate of rise of the action potential.

Nifedipine caused similar reductions in the force of contraction and changes in the action potential configuration to those caused by oxmetidine and verapamil but was active over the dose range 1×10^{-8} to 1×10^{-6}M (Figures 2 and 3).

The changes in action potential configuration at comparable negative inotropic concentrations of the three agents were similar (Table 1).

Studies on force of contraction and action potential configuration in partially depolarized preparations

Oxmetidine reduced the force of contraction and changed the configuration of the Ca^{2+}-dependent

Table 1 Changes in the force of contraction and action potential configuration in guinea-pig papillary muscle preparations caused by oxmetidine, verapamil and nifedipine

		% change from control				
Drug	*Concentration* (M)	*Force of contraction*	APD_{20}	APD_{90}	*APM*	dV/dt_{max}
Oxmetidine ($n = 5$)	1×10^{-4}	−49.5 ± 3.0	−7.9 ± 3.7	−2.9 ± 1.7	−4.6 ± 3.2	−3.4 ± 9.5
Verapamil ($n = 3$)	1×10^{-5}	−51.2 ± 6.6	−6.5 ± 2.5	−4.7 ± 2.6	−1.8 ± 0.4	−2.7 ± 5.5
Nifedipine ($n = 3$)	3.16×10^{-7}	−58.9 ± 2.8	−13.7 ± 1.7	−8.7 ± 2.3	−1.0 ± 0.3	−0.2 ± 2.0

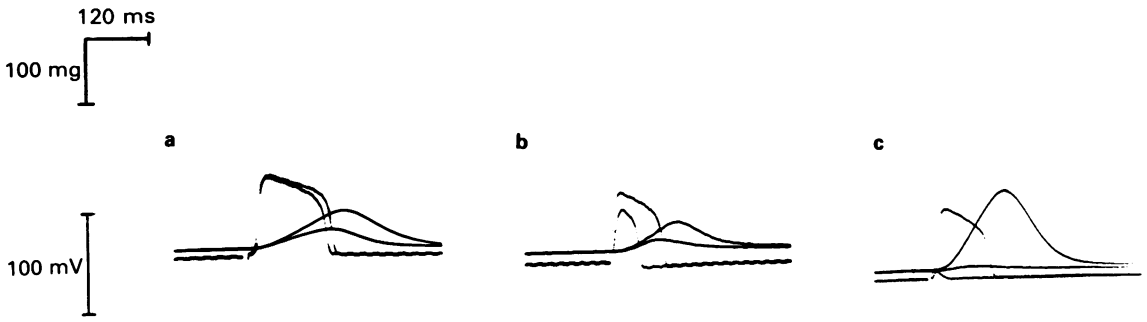

Figure 4 Guinea-pig papillary muscle, contraction and action potential in partially depolarized preparations (27 mM K^+). Treatment with (a) oxmetidine, 1×10^{-4}M, (b) verapamil, 1×10^{-5}M, or (c) nifedipine, 3.16×10^{-7}M, reduces the force of contraction and the action potential configuration. In each record, the upper contraction curve is the control response and the lower curve is that in the presence of drug. The outer action potential is the control and the inner action potential that in the presence of drug. In all cases, drug exposure was for 15 min.

action potential in preparations partially depolarized with K^+, 27 mM (Figures 4 and 5). The inhibition of contraction caused by oxmetidine, 1×10^{-4}M ($n = 3$), and 3.16×10^{-5}M ($n = 1$), was similar, expressed as percentage inhibition of control response, in normal Krebs Henseleit solution and in that with raised K^+ to cause partial depolarization (Figures 4 and 5).

Verapamil was far more effective as a negative inotropic agent in partially depolarized preparations than in normal preparations (Figures 4 and 5); the concentration to reduce the force of contraction by 50% was decreased from approximately 1×10^{-5}M in normal Krebs solution to between 3.16×10^{-7} and 1×10^{-6}M in partially depolarized preparations (Figure 5).

Nifedipine, 3.16×10^{-7}M, was also more effective as a negative inotropic agent in partially depolarized preparations (Figures 4 and 5).

The changes in action potential magnitude and the maximum rate of rise of the action potential for comparable negative inotropic concentrations of oxmetidine and verapamil are shown in Table 2.

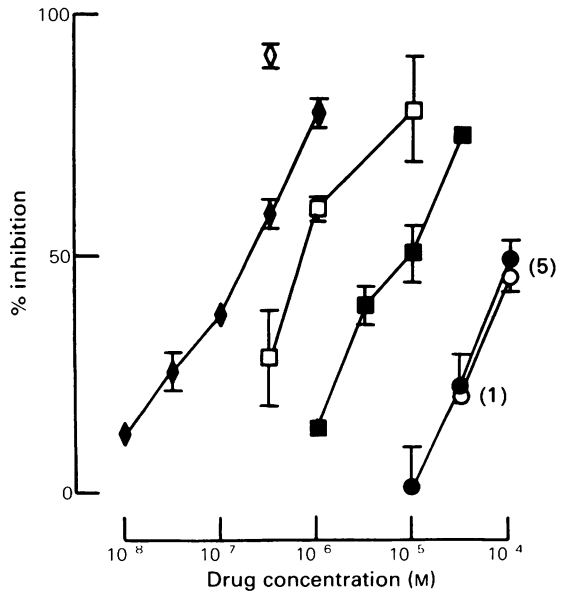

Figure 5 Inhibition of guinea-pig papillary muscle contraction by nifedipine (◆,◇), verapamil (■,□) and oxmetidine (●,○). Responses in Krebs Henseleit buffer, 5.9 mM K^+, are shown by filled symbols, responses in Krebs Henseleit buffer, 27 mM K^+, are shown by open symbols. Values are mean with s.e.mean shown by vertical lines; $n = 3$ except where marked.

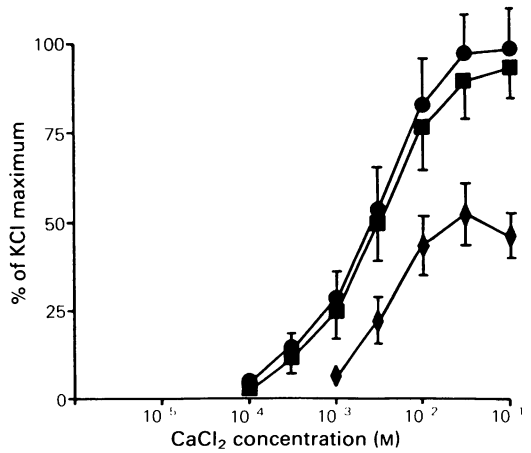

Figure 6 Contraction of dog saphenous vein by increasing calcium concentration. Concentration-response curve to calcium in the absence of oxmetidine (●) and in the presence of oxmetidine, 1×10^{-5}M (■), or 1×10^{-4}M (◆). Points are mean with s.e.mean shown by vertical lines; $n = 4$.

Table 2 Changes in the force of contraction and action potential configuration caused by oxmetidine and verapamil in partially depolarized (27 mM K^+) guinea-pig papillary muscles

		% change from control			
Drug	*Concentration* (M)	*Force of contraction*	*APM*	dV/dt_{max}	APD_{90}
Oxmetidine ($n = 3$)	1×10^{-4}	−45.9 ± 3.0	− 4.4 ± 1.9	−10.0 ± 2.8	− 4.1 ± 0.7
Verapamil($n = 3$)	3.16×10^{-7}	−28.7 ± 10.0	− 4.7 ± 1.1	−22.1 ± 5.0	− 9.5 ± 3.5
	1×10^{-6}	−60.6 ± 2.1	−17.9 ± 4.9	−48.2 ± 4.7	−35.7 ± 11.6

Verapamil had a relatively greater inhibitory effect on the action potential than oxmetidine at equivalent negative inotropic concentrations.

Studies in dog saphenous vein preparations

Inhibition of KCl-induced calcium contraction Oxmetidine, 1×10^{-5}M, had no effect on calcium-induced contractions of dog saphenous vein but caused substantial reduction of responses at 1×10^{-4}M (Figure 6).

Studies with nifedipine and verapamil showed that equivalent inhibition of calcium-induced contractions occurred using nifedipine, 1×10^{-9}M, verapamil, 1×10^{-7}M and oxmetidine, 1×10^{-4}M.

$^{45}Ca^{2+}$-uptake studies Oxmetidine, at concentrations up to 5×10^{-4}M, did not affect basal $^{45}Ca^{2+}$-uptake in dog saphenous vein. At 1×10^{-3}M, oxmetidine reduced this basal inhibition by 75%, (data not shown).

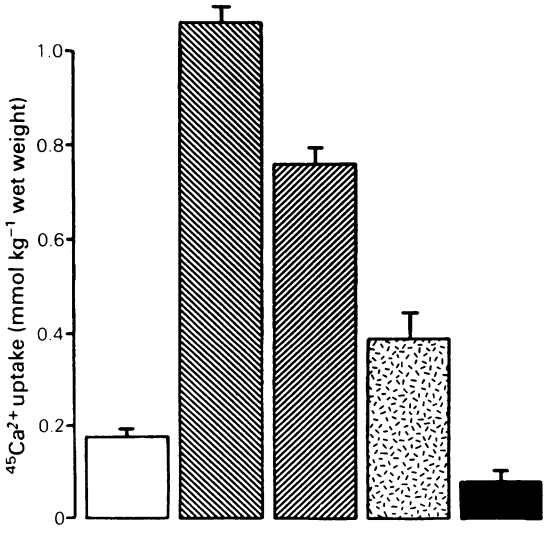

Figure 7 Uptake of $^{45}Ca^{2+}$ by dog saphenous vein. Basal uptake (□), uptake in the presence of 80 mM KCl (▧) which is inhibited by oxmetidine, 1×10^{-4}M (▨), 5×10^{-4}M (▨) and reduced to basal uptake by oxmetidine, 1×10^{-3}M (■). Values are mean with s.e.mean shown by vertical lines; $n = 7-10$ per treatment.

Depolarization of the preparations by addition of KCl, 80 mM, produced a net increase in $^{45}Ca^{2+}$-uptake of 0.89 ± 0.02 mmol kg^{-1} wet weight. This increase in $^{45}Ca^{2+}$-uptake was inhibited, in a concentration-dependent manner, by oxmetidine 10^{-4} to 10^{-3}M (Figure 7), the concentration of oxmetidine to reduce $^{45}Ca^{2+}$-uptake by 50% was approximately 2×10^{-4}M. In similar studies, verapamil reduced $^{45}Ca^{2+}$-uptake over the concentration range 1×10^{-7} to 1×10^{-5}M, the concentration to reduce uptake by 50% was approximately 4×10^{-7}M.

Haemodynamic studies in anaesthetized cats

Oxmetidine had no consistent effect on any haemodynamic parameter when infused at 8×10^{-8} or 4×10^{-7} mol kg^{-1} min^{-1}. Small statistically significant falls in blood pressure, heart rate and total peripheral resistance occurred during infusion at 2×10^{-6} mol kg^{-1} min^{-1}; larger significant falls in these parameters occurred during infusions at 1×10^{-5} mol kg^{-1} min^{-1}. There were no significant changes in stroke volume at any of the doses of oxmetidine used. The results are illustrated in Figure 8.

Discussion

Oxmetidine is a potent histamine H_2-receptor antagonist, active at concentrations of 1×10^{-7}M and above (Blakemore *et al.*, 1980). Higher concentrations of oxmetidine have been shown to elicit negative actions on cardiac preparations from various species in a manner which suggested that oxmetidine might interfere with the transmembrane transport of calcium (Coruzzi *et al.*, 1983). The present study confirms that oxmetidine has negative inotropic actions and additionally causes blood vessel dilatation at concentrations higher than those needed for H_2-receptor antagonism. Analysis of these actions is consistent with an effect of oxmetidine on the slow inward calcium current although, in some conditions, the nature of this action could be readily distinguished from that of verapamil or nifedipine.

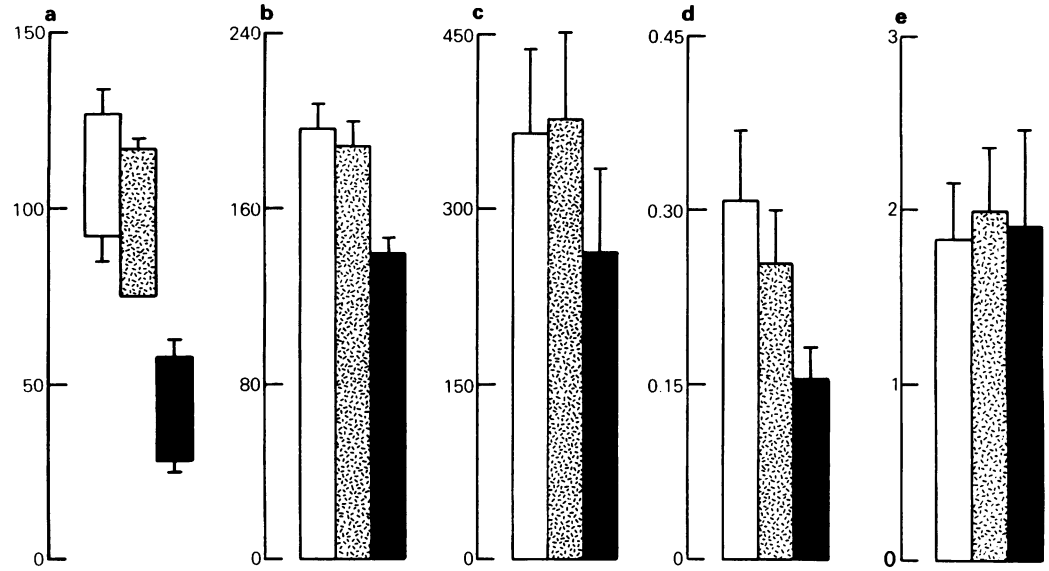

Figure 8 Haemodynamics in anaesthetized cats. Changes in (a) blood pressure (mmHg); (b) heart rate (beats min^{-1}); (c) aortic blood flow, (ml min^{-1}); (d) total peripheral resistance (units) and (e) stroke volume (ml) caused by oxmetidine. Control values are shown by open histograms, values during infusion of oxmetidine, 2×10^{-6} mol kg^{-1} min^{-1} by stippled histograms and during infusion of oxmetidine, 1×10^{-5} mol kg^{-1} min^{-1} by solid columns. Values are mean with s.e.mean shown by vertical lines; $n = 3$.

In isolated working heart preparations, oxmetidine caused negative inotropic and chronotropic actions over the same concentration-range, i.e. in excess of 1×10^{-6}M. These findings are comparable to those described by Coruzzi *et al.* (1983) in separate atrial and ventricular preparations from guinea-pigs or in Langendorff heart preparations from rabbits. Oxmetidine also reduced cardiac output and stroke volume over the same concentration-range but did not alter coronary flow.

In contrast, under the experimental conditions used, verapamil showed clear selectivity in causing a negative inotropic response with only minimal bradycardia. Stroke volume was reduced as was cardiac output at concentrations with minimal effects on heart rate. Like oxmetidine, verapamil had little effect on coronary flow. Maintenance of coronary flow with both agents despite the significant reduction in cardiac work implies that each agent caused coronary vasodilatation although this was not examined more directly.

Studies on guinea-pig papillary muscles showed that the negative inotropic activity of oxmetidine was associated with a shortening in the plateau phase of the action potential, a change consistent with a reduction in the Ca^{2+}-carried second slow inward current of the action potential (Reuter & Scholz, 1977; Reuter, 1979). At equivalent negative inotropic concentrations, the changes in action potential configuration caused by oxmetidine were similar to those caused by either verapamil or nifedipine, two compounds shown to inhibit the slow inward Ca^{2+}-dependent current by a wide variety of techniques, further indicating that the negative inotropic actions of oxmetidine were due to inhibition of the Ca^{2+}-dependent current. Unexpectedly, at the highest concentration of oxmetidine used, 3.16×10^{-4}M, the negative inotropic action was partly and variably reversed although there was further shortening of the plateau phase of the action potential. The nature of this reversal of the negative inotropic response, and its apparent dissociation from further reduction in the slow Ca^{2+}-dependent current is a potentially interesting phenomenon which has not been further investigated.

Further evidence that oxmetidine inhibits the slow Ca^{2+}-dependent current was derived from studies in partially depolarized preparations in which the fast Na^{+}-dependent current is inactivated and the action potential upstroke velocity and magnitude are largely dependent on the Ca^{2+} current. Oxmetidine showed similar negative inotropic activity to that in normal Krebs solution and caused a reduction in both the upstroke velocity and magnitude of the Ca^{2+}-dependent action potential. These studies in partially depolarized preparations however revealed an important difference between oxmetidine and Ca^{2+} antagonists such as verapamil, nifedipine and diltiazem. Thus, it is recognised that verapamil effects are both voltage- and

use-dependent (Ehara & Kaufmann, 1978; Kohlhardt & Mnich, 1978). Similar findings have been obtained with diltiazem (Tung & Morad, 1983; Lee & Tsien, 1983) whereas findings have been less clear with dihydropyridine antagonists, such as nifedipine, nisoldipine, nitrendipine and nicardipine, which have variously been shown to exhibit little voltage-dependence (e.g. Kohlhardt & Fleckenstein, 1977; Bayer & Ehara, 1978; Tung & Morad, 1983) or, in a recent study in calf Purkinje fibres using a voltage clamp technique, to show clear voltage-dependence (Sanguinetti & Kass, 1984). The results in the present study confirm the voltage-dependent responses to verapamil and to the dihydropyridine, nifedipine. Oxmetidine thus clearly differs from these Ca^{2+} antagonists in its lack of voltage-dependence as a negative inotropic agent. Further, at equivalent negative inotropic concentrations in partially depolarized preparations, although oxmetidine reduced Ca^{2+}-dependent action potentials, the reduction was less than that in muscles treated with verapamil.

Studies on vascular tissue, dog saphenous vein preparations, also produced data consistent with oxmetidine acting to reduce the Ca^{2+}-dependent current. Data was obtained in preparations depolarized by raising extracellular K^+ and showed that Ca^{2+}-dependent contractions and $^{45}Ca^{2+}$-uptake were inhibited by oxmetidine at 10^{-4}M, similar to the concentration used in the papillary muscle studies. In these studies, in depolarized preparations, verapamil was approximately 1000 times more potent than oxmetidine and nifedipine a further 100 times more potent than verapamil. The reduction in Ca^{2+}-induced responses by oxmetidine was paralleled by reduction in $^{45}Ca^{2+}$-uptake. The action of oxmetidine was clearly demonstrated in the presence of high extracellular K^+ at concentrations that did not reduce the basal 'leak' uptake of $^{45}Ca^{2+}$ in studies with normal (5 mM) K^+.

The haemodynamic consequences of these actions of oxmetidine were studied in anaesthetized cats. The vasodilator action of oxmetidine was shown by the dose-dependent reductions in peripheral vascular resistance and blood pressure and actions on the heart were reflected by bradycardia. Oxmetidine had no effect on cardiac output, at doses which caused small reductions in heart rate and peripheral resistance presumably as any negative inotropic action tending to reduce output was balanced by the reduction in afterload which would tend to increase cardiac output. At the higher infusion rate, the reduction in cardiac output presumably reflected a marked negative inotropic action, the consequences of which exceed the consequences of reduced afterload.

In conclusion, oxmetidine, at concentrations in excess of those needed to cause H_2-receptor antagonism and which exceed those found in therapeutic studies, elicited negative inotropic activity and caused vasodilatation. These actions of oxmetidine were associated with a reduction of the slow inward Ca^{2+}-dependent current assessed either by electrophysiological techniques or by measurement of $^{45}Ca^{2+}$-uptake. Unlike other calcium antagonists examined, oxmetidine did not show voltage-dependent activity in ventricular muscle. Further studies will be required to define better the nature of the action of oxmetidine on the slow Ca^{2+}-dependent current in ventricular and vascular muscle; these results however suggest a novel interaction between oxmetidine and transmembrane Ca^{2+} flux.

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