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An in vitro study of the effects of calcium on the cardiovascular actions of thiopentone, Althesin and ketamine in the rat

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The actions of three intravenous anaesthetics, Althesin, thiopentone and ketamine have been compared on the rat isolated atria and portal vein. Although the three anaesthetics had grossly similar actions on the two preparations. i.e. depression of atrial rate and depression of the amplitude of myogenic activity in the portal vein, there were enough differences to suggest that they produced their effects by different mechanisms. These differences were particularly obvious in interactions with noradrenaline and the effects of changes in calcium ion concentration on the concentration effect relationships for the agents on the atria and portal vein. Generally Althesin was unaffected by changes in calcium ion concentration, whilst ketamine and thiopentone were affected, but in qualitatively different ways.

Intravenous anaesthetics commonly affect the cardiovascular system. The barbiturate anaesthetics cause a fall in blood pressure (Conway & Ellis 1969) and direct myocardial depression by the barbiturates has been convincingly demonstrated (Dundee & Wyant 1974). In contrast, ketamine has been shown to increase heart rate and blood pressure (Savege et al 1976). Like the barbiturates, Althesin causes a fall in blood pressure (Savege et al 1972) but this is usually associated with an increase in heart rate (Savege et al 1971). Whilst animal experiments have shown that Althesin has a negative inotropic effect, this is only seen with high doses (Iwatsuki 1973).

A role of calcium in the various cardiovascular actions of some anaesthetics has been proposed. Barbiturates have been shown to mimic the effects of calcium withdrawal on the heart (Fleckenstein 1964), whilst the similarity between the actions of ketamine and added calcium ion has been remarked upon by Johnstone (1976).

Thus it is possible that changes in extracellular calcium concentration could alter the cardiovascular actions of these anaesthetic agents.

The present study was designed to investigate this possibility in vitro.

MATERIALS AND METHODS

Female Sprague-Dawley rats (150 to 250 g) were killed by a blow on the head and the portal vein and atria were dissected out. The bathing fluid for the isolated preparations was either single, double or triple calcium Krebs-Henseleit solution mM (NaCl 118: KCl 4·75; $MgSO_4$, $7H_2O$ 1·2; KH_2PO_4 1·19; $NaHCO_3$ 25; glucose 5·55 and either 2·55, 5·1 or 7·65 $CaCl_2$ $6H_2O$). The fluid was maintained at 37 °C and bubbled with 5% CO_2 in oxygen. If the triple calcium solution was left standing for several hours, some haziness occassionally occurred, so this solution was freshly prepared several times during an experimental day.

Atria

All preparations were set up initially in double calcium Krebs-Henseleit solution under a tension of 0·5 g. Rat myocardium requires 5mM $CaCl_2$ for good contractility and stability (Katzung 1968). Changes in tension were recorded using an Ether strain gauge transducer connected to a Grass 79C polygraph fitted with a tachograph triggered from the tension recorder. Paced preparations were stimulated at 5 Hz with supramaximal voltage and 1·5 ms pulse width. Modified Krebs-Henseleit solutions were added after the preparations had stabilized.

Using a 10 min contact time, concentrations of anaesthetic were added in a random sequence and only one anaesthetic was tested on each preparation. Maximum responses to the anaesthetics were obtained 3–5 min after the addition of the drug.

The effects of the anaesthetics on noradrenaline responses were examined on preparations bathed in double calcium Krebs-Henseleit solution. Cumulative concentration effect curves for noradrenaline were obtained with a 2 min interval between additions of noradrenaline. Preparations were pretreated

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with three concentrations of anaesthetic for 10 min before adding noradrenaline.

Hepatic portal veins

The portal veins were set up initially in single calcium Krebs-Henseleit solution under a tension of 1 g. Myogenic activity and responses to noradrenaline were recorded using an Ether strain gauge transducer connected to a Rikadenki B-104 recorder.

As with the atria, modified Krebs-Henseleit solutions were added after stabilization. The effects of the anaesthetics upon the myogenic activity of the portal vein were examined using a 10 min contact time. Only one anaesthetic was used on each preparation and concentrations were added in a random sequence. Cumulative concentration effect curves for noradrenaline were obtained on preparations bathed in single calcium Krebs-Henseleit solution. The anaesthetics were added 10 min before the noradrenaline.

Drugs used

Thiopentone sodium 2·5 to 160 μg ml^{-1} (solutions freshly prepared daily); Althesin 0·3 to 307·2 μg ml^{-1} total steroid (prepared from the commercial product); ketamine hydrochloride 2·5 μg ml^{-1} to 1·2 mg ml^{-1}; polyoxyethylated castor oil (Cremophor EL) in dilutions equivalent to those in Althesin; alphaxalone and alphadolone in concentrations equivalent to those in Althesin (solubility permitting); noradrenaline bitartrate 5 ng ml^{-1} to 80 μg ml^{-1}; verapamil 2 μg ml^{-1} and sodium nitroprusside 0·5 to 50 μg ml^{-1}.

Generally results are expressed as means and standard errors of not less than 5 experiments. Significance was assessed using the Students *t*-test or, when this was not applicable the Mann Whitney U-test.

RESULTS

Rat atria

Thiopentone, Althesin and ketamine, all caused a concentration-dependent fall in atrial rate. No increase in atrial rate was seen with any of the concentrations of anaesthetics used. The log EC50 values for depression of atrial rate of the three agents in the three calcium concentrations are shown in Table 1. Only the log EC50 for thiopentone was significantly affected by changes in calcium concentration. The whole concentration effect relationship for thiopentone was displaced to the left by increasing calcium concentration (Fig. 1).

In spontaneously beating preparations all three anaesthetics caused a variable, but significant, increase in the amplitude of contraction. However in paced preparations only two anaesthetics, thiopentone and ketamine caused a significant increase in amplitude. The response of the atria to these two agents was biphasic, the initial increase in tension being followed by a decrease. The maximum increase in tension with thiopentone, in double calcium Krebs-Henseleit solution, was obtained with 320 μg ml^{-1} (+16·1 ± 5·1%), but at this concentration the preparations subsequently failed to contract. Ketamine 160 μg ml^{-1}, caused a similar increase in amplitude of contraction (15·4 ± 6·3%), but subsequent decreases in amplitude were relatively small (14·1 ± 5·9%). Concentrations of Althesin above 9·2 μg ml^{-1} caused a concentration dependent decrease in the tension of the paced atria.

Changing the calcium concentration in the fluid bathing the paced atria did not significantly alter the responses of the preparations to the anaesthetics although the depressant actions of thiopentone appeared to be greater in the triple calcium solution.

Table 1. Effect of calcium concentration on the depression of rat atrial rate produced by anaesthetics in vitro.

	Log EC50 (μg ml^{-1}) Calcium concentration in Krebs-Henseleit solution		
	Single	Double	Triple
Thiopentone	1·75 ± 0·05	1·56 ± 0·10	1·35 ± 0·07*
Althesin	1·38 ± 0·18	1·21 ± 0·18	1·25 ± 0·02
Ketamine	2·07 ± 0·06	2·06 ± 0·14	2·18 ± 0·14

Althesin concentration is expressed as total steroid.
* $P < 0·05$ compared to single calcium values.
Results are expressed as means ± standard error of 5 experiments.

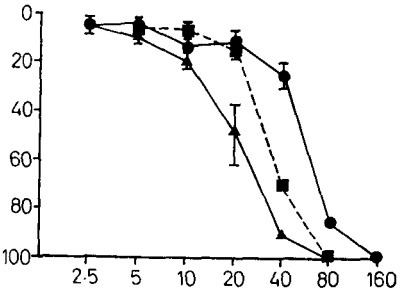

FIG. 1. Effect of changes in calcium concentration on the depression of rat atrial rate produced by thiopentone in vitro. Each point is the mean ± standard error of 5 experiments. (●) single calcium, (■) double calcium, (▲) triple calcium Krebs-Henseleit solution. Ordinate: depression of atrial rate (%). Abscissa: thiopentone sodium (μg ml^{-1}).

Analysis of the effects of the anaesthetics upon the response of the atria to noradrenaline is confused by the depression of atrial rate produced by these agents. Cumulative concentration effect curves to noradrenaline were unaffected by concentrations of anaesthetics which did not cause a fall in atrial rate.

However, it was possible to demonstrate a difference between the effects of the anaesthetics upon the atrial response to noradrenaline. The maximum increase in atrial rate in response to noradrenaline was 73 ± 15 beats min^{-1} before 2·4 $\mu g\ ml^{-1}$ Althesin and 71 ± 4 beats min^{-1} in its presence. This concentration of Althesin depressed atrial rate by 16·6 ± 3·3%. In contrast, in the presence of 20 $\mu g\ ml^{-1}$ thiopentone, which caused a similar depression of atrial rate (16·7 ± 3·4%) the maximum increase in atrial rate produced by noradrenaline was increased to 189 ± 18 beats min^{-1} from a pre-thiopentone value of 120 ± 9 beats min^{-1}. The enhancement of noradrenaline response by thiopentone increased with increasing concentration of thiopentone. Ketamine affected the response of the atria to noradrenaline in a qualitatively similar manner to thiopentone although the increase in noradrenaline maximum (from 79 ± 13 to 111 ± 19 beats min^{-1}) produced by 20 $\mu g\ ml^{-1}$ ketamine was not significant. This concentration of ketamine reduced atrial rate by 11·4 ± 1·8%.

Verapamil, 50 ng ml^{-1}, reduced atrial rate by 23·0 ± 2·5% and, like thiopentone, increased the maximum increase in atrial rate produced by noradrenaline from 84·0 ± 11 beats min^{-1} to 156 ± 12 beats min^{-1}. Sodium nitroprusside in the concentrations used had no effect either on atrial rate or response to noradrenaline.

Only thiopentone, in high concentrations, changed the pH of the Krebs-Henseleit solution. This was a small but significant increase in pH. However, control experiments showed that similar changes in pH produced by the addition of a trace of sodium hydroxide had no significant effect on either the amplitude or rate of atrial contractions. Furthermore, neither sodium hydroxide nor thiopentone caused any significant calcium precipitation even in the triple calcium solution. Cremophor EL in concentrations similar to those in Althesin increased the variability of the atrial trace, but did not produce significant changes in either tension or atrial rate. All changes which were obtained with alphaxalone and alphadolone could be reproduced by dilution of the Krebs-Henseleit solution with the large volumes of vehicle necessary to dissolve these compounds.

Rat portal vein

All three anaesthetics caused a concentration dependent decrease in the amplitude of the myogenic activity of this preparation. The log ED50 values for the anaesthetics and the slopes of the concentration effect relationships in the three calcium concentrations are shown in Table 2. Only Althesin produced the same quantitative effects in all three calcium concentrations. The slope of the thiopentone concentration effect relationship became progressively steeper as the calcium concentration increased although the ED50 value was not significantly altered. In contrast, the slope for the ketamine relationship was unaltered by increasing calcium concentration, but the whole curve was progressively shifted to the right. This is illustrated in Fig. 2 which also shows that low concentrations of ketamine, in single calcium Krebs-Henseleit solution significantly increased the amplitude of the myogenic activity. This enhancement of myogenic activity was abolished in double and triple calcium Krebs-Henseleit solution.

The frequency of the myogenic activity of the portal vein was increased by all anaesthetics and this became progressively greater as the amplitude of the

Table 2. Effect of calcium concentration on the depression of amplitude of myogenic activity produced by anaesthetics on the rat portal vein in vitro.

	Calcium concentration in Krebs-Henseleit solution		
Log EC50 ($\mu g\ ml^{-1}$)	Single	Double	Triple
Thiopentone	1·85 ± 0·04	2·06 ± 0·13	2·03 ± 0·09
Ketamine	2·08 ± 0·03	2·20 ± 0·08	2·32 ± 0·06*
Althesin	1·53 ± 0·14	1·70 ± 0·11	1·63 ± 0·22
Slope ($1/\mu g\ ml^{-1}$)			
Thiopentone	—0·0205 ± 0·0014	—0·0152 ± 0·0003*	—0·0114 ± 0·0007*
Ketamine	—0·0057 ± 0·0006	—0·0087 ± 0·0017	—0·0061 ± 0·0008
Althesin	—0·0235 ± 0·0043	—0·0165 ± 0·0026	—0·0150 ± 0·0036

Althesin concentrations are expressed as total steroid.
* $P < 0·05$ compared to single calcium values.
Results are expressed as means ± standard errors of not less than 5 experiments.

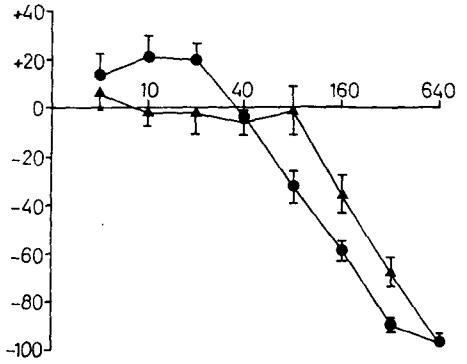

FIG. 2. The effects of ketamine (abscissa: μg ml^{-1}) on the amplitude of myogenic activity in the rat portal vein bathed in either single (●) or triple (▲) calcium Krebs-Henseleit solution. Each point is the mean ± standard error of not less than 5 experiments. Ordinate: change in amplitude %).

activity decreased. When amplitude was more than 60% depressed, the frequency of the activity became so great as to be unmeasurable.

In single calcium Krebs-Henseleit solution the relationship between depression of amplitude and enhancement of frequency of myogenic activity was similar for all three anaesthetics. However, in double and triple calcium solutions the relationship was unchanged for Althesin but displaced to the left for ketamine and thiopentone. The relationship for thiopentone in single and double calcium solutions is shown in Fig. 3. The control frequency of myogenic activity was also significantly different in these two calcium solutions ($P < 0.05$). In double calcium solution the frequency was 2·06 ± 0·20 contractions min^{-1} whilst in single calcium solution it was 3·4 ± 0·20 contractions min^{-1}.

The response of the rat portal vein to cumulative concentrations of noradrenaline was reduced by all three agents. There was no rightward shift of the concentration effect relationship, but the maximum response was progressively reduced by increasing concentrations of the anaesthetics.

Althesin depressed the noradrenaline maximum in concentrations which caused a similar percentage reduction in the amplitude of the myogenic activity. However this was not the case for thiopentone and ketamine, which significantly depressed the maximum response to noradrenaline in concentrations which had no effect upon the amplitude of the myogenic activity. For example, in single calcium Krebs-Henseleit solution ketamine (40 μg ml^{-1}) depressed the noradrenaline maximum response by 39·0 ± 6·3%, whilst the change in amplitude of myogenic

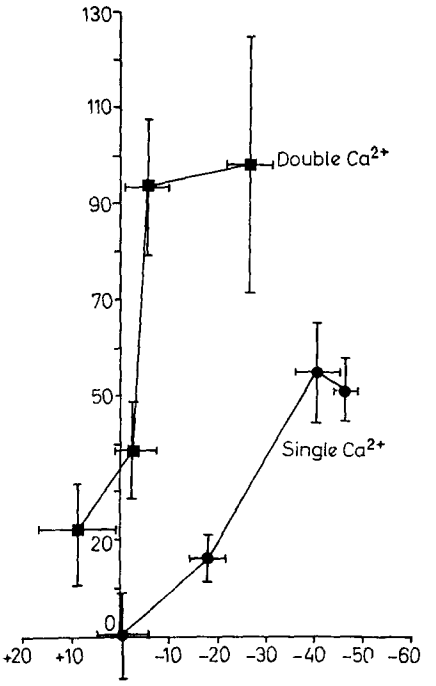

FIG. 3. The relationship for thiopentone between depression of amplitude and depression of frequency of myogenic activity in single (●) and double (■) calcium Krebs Henseleit solution. Results are means ± standard error of not less than 5 experiments. Ordinate: change of frequency (%). Abscissa: △ amplitude (%).

activity produced by this concentration of ketamine was +1·85 ± 7·6%. Similarly thiopentone 10 μg ml^{-1} depressed the maximum response to noradrenaline by 51·0 ± 6·7% whilst depressing myogenic activity by only 0·5 ± 4·8%. The greater selectivity of thiopentone and ketamine for depression of the noradrenaline responses occurred throughout the concentration ranges used in this study.

The results for the intravenous anaesthetics were compared with those of two other smooth muscle relaxants, verapamil and sodium nitroprusside. Verapamil 2 μg ml^{-1} caused a 37·1 ± 4·1% decrease in amplitude of myogenic activity whilst depressing the noradrenaline maximum by 86·7 ± 4·1%. Thus, like thiopentone and ketamine, verapamil produces greater depression of noradrenaline responses. In contrast sodium nitroprusside 0·5 μg ml^{-1} caused a 33·6 ± 11·0% depression of myogenic amplitude but the noradrenaline maximum response was only depressed by 12·0 ± 4·7%.

As with the atrial preparation, pH changes equivalent to the highest concentrations of thiopentone, cremophor EL and alphaxalone and

alphadolone in dilutions which did not significantly dilute the Krebs-Henseleit solution, produced no significant changes in the portal vein preparation.

DISCUSSION

There were a number of qualitative similarities in the effects of the three anaesthetics on the rat atria and portal vein. All the anaesthetics caused a concentration-dependent decrease in atrial rate and the amplitude of the myogenic activity of the portal vein, the latter effect being coupled with an increase in the frequency of myogenic activity. None of the anaesthetics depressed the response of the atria to noradrenaline, but all three agents depressed the response of the portal vein to noradrenaline.

However, in detail, there were differences between the anaesthetics. In low concentrations, ketamine caused an increase in the amplitude of the myogenic activity produced by the portal vein in single calcium Krebs-Henseleit solution. This action of ketamine, which could contribute to the increase in blood pressure produced by this drug, was completely abolished by raising the calcium concentration.

On the paced atrial preparations, ketamine and thiopentone produced a biphasic response; an initial increase in tension followed by a decrease. Althesin, however, only caused a decrease thus confirming the observations of Iwatsuki (1973). A biphasic effect of ketamine and another barbiturate, methohexitone, on the tension of skeletal muscle has been reported (Kraunak et al 1977). The enhancement of twitch tension was demonstrated in curarized preparations and was a property not shared by Althesin. Thus, it would seem that there is a basic qualitative difference between the actions of Althesin on the one hand and ketamine and barbiturates on the other with respect to more than one muscle system.

Althesin also behaved in a qualitatively different manner to thiopentone and ketamine when relationships between depression of spontaneous activity and effect on noradrenaline responses were examined. Althesin showed no marked selectivity of action, whilst thiopentone and ketamine preferentially depressed noradrenaline responses on the portal vein and spontaneous activity on the atrial preparation. In this respect thiopentone and ketamine resembled the actions of verapamil on these two preparations. Verapamil has been reported to slow the heart, whilst having little effect on noradrenaline responses by 'calcium antagonism' (Nayler & Krikler 1974). Furthermore, in smooth muscle preparations, verapamil and related compounds are believed to preferentially inhibit one of at least two separate calcium activating systems (Kreye et al 1975) thus inhibiting myogenic activity and reducing responses to noradrenaline (Jetley & Weston 1976).

The involvement of calcium in the slowing of the atria by thiopentone is also possible, as alteration of the calcium content of the bathing fluid significantly changed the sensitivity of the preparation to the depressant actions of thiopentone. Nayler & Szeto (1972) demonstrated that pentobarbitone increased the time required by cardiac muscle for relaxation and that this appeared to be associated with impaired removal of Ca^{2+} from the intracellular fluid by the sarcoplasmic reticulum. Thus the barbiturate induced increase in recovery time, leading to slowing of the rate of contraction, is due to the presence of excess Ca^{2+} within the cell. Removal of this excess calcium would be more difficult if the extracellular calcium levels were raised. This could explain the increased sensitivity of the atria to thiopentone induced rate depression in triple calcium Krebs-Henseleit solution.

However, the interaction between thiopentone and calcium may not relate directly to thiopentone's mechanism of action as calcium may also affect the binding of the drug to the tissue. This has been demonstrated experimentally for the local anaesthetics (Blaustein & Goldman 1966) although the binding of phenytoin, a drug chemically related to the barbiturates, is not affected by calcium concentration (Goldberg 1977).

The actions of ketamine and Althesin on the atria were unaffected by changes in calcium concentration. Whilst this does not rule out a role of calcium in their actions, it does suggest that the biochemical mechanism of their actions or binding is distinct from that of thiopentone on this tissue.

Both the actions of ketamine and thiopentone on the portal vein were affected by the calcium concentration of the bathing fluid. The frequency of the myogenic activity of the portal vein was itself decreased by increased calcium concentration. This effect of calcium was reversed by both ketamine and thiopentone in concentrations which had no intrinsic effect upon the myogenic activity of the portal vein in low calcium solutions. Althesin again differed from the other two agents in that it did not antagonize this action of calcium.

Calcium concentration also affected the portal veins sensitivity to ketamine and thiopentone. This was evident as a change in slope of the concentration effect relationship for thiopentone and a parallel displacement for ketamine. It is clear, not only from

the work with specific calcium antagonists, but also from the effects of calcium concentration on a number of vasoconstrictor agents (Hudgins & Weiss 1968) that there are a number of stores which can be affected by drugs and it may be that thiopentone affects a different one from ketamine. Furthermore, as mentioned above, the effects of calcium on the binding of the drug to the tissue may also be relevant.

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