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An In-vitro Study of the Interactions Between Intravenous Induction Agents and the Calcium Antagonists Verapamil and Nifedipine

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Abstract—Thiopentone, propofol and etomidate inhibit the contractions of the rat isolated atria and portal vein. The actions of thiopentone and propofol summate with those of verapamil and nifedipine. Verapamil potentiates the action of etomidate on both preparations. The depressant actions of thiopentone and propofol on the portal vein are associated with a reduced response to calcium. Etomidate does not reduce the response to calcium in this preparation.

The cardiovascular effects of both verapamil and nifedipine can be explained by the ability to block calcium entry to the cell (Godfraind et al 1986). It has been suggested that calcium has some role in the cardiovascular responses to anaesthetic agents. Halothane has been shown by Lynch et al (1980) to depress the slow action potential of the heart. Several workers have now demonstrated an interaction between the volatile anaesthetic agents, halothane, enflurane and isoflurane and the calcium antagonists nifedipine, diltiazem and verapamil (cf. Reves et al 1982). Whilst, in general, interactions between inhalational agents and calcium antagonists are additive (cf. Lynch 1988) some reports have demonstrated potentiation with particular combinations of agents. On the guinea-pig isolated perfused heart Marijic et al (1988) showed that verapamil produced auricular-ventricular (AV) block and decreased cardiac function in the presence of enflurane but not halothane or isoflurane.

Fleckenstein (1964) observed that barbiturates mimic the effect of calcium withdrawal on the heart and Godfraind et al (1986) classified barbiturates as non-selective calcium antagonists. This suggests that calcium antagonism may be involved in the cardiovascular depression seen when barbiturates are used as intravenous anaesthetic agents. Propofol, a recently introduced intravenous anaesthetic agent also reduces arterial blood pressure principally by decreasing systemic vascular resistance (Patrick et al 1985). In contrast, etomidate, an intravenous anaesthetic agent, is considered less likely to cause cardiovascular depression in clinical doses (Gooding et al 1979) although higher doses do decrease cardiac output and arterial pressure (Prakash et al 1981). However, there is little information in the literature concerning the possible mechanisms of the cardiovascular effects of propofol and etomidate or whether significant interactions with calcium antagonists might be expected.

The present study aims to obtain information concerning these effects by the use of two simple in-vitro preparations, the rat isolated atria and portal vein.

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Materials and Methods

Male Sprague-Dawley rats (150–250 g) were killed, the portal vein and atria dissected out and suspended in Krebs-Henseleit solution through which 95% oxygen, 5% carbon dioxide was bubbled in a 20 mL tissue bath maintained at 37°. Once the tissue had been attached to an Ether strain gauge by cotton thread, the tension was adjusted to 1 g for vein and 0·5g for atria. Contractions were recorded on a potentiometric recorder (Rikadenki B-104) for the vein and a Grass 79C polygraph fitted with a tachograph triggered from the tension record for the atria.

The preparations were allowed to settle for approximately 30 mins and a steady state contraction amplitude was recorded. Either verapamil (5×10^{-8} to 4×10^{-7}M) or nifedipine (10^{-11} to 10^{-7} M) was then added to the bath. Preliminary experiments in which the calcium antagonists were added to the bath for 2 h demonstrated that maximal depression occurred between 10 and 30 min after exposure and thereafter the tension of contractions remained constant. For example, the depression of contractions after 30 min exposure to 4×10^{-7}M verapamil was $42{\cdot}8 \pm 3{\cdot}3\%$ and after 120 min $45{\cdot}6 \pm 5{\cdot}0\%$. Once maximal depression was seen, a cumulative concentration response relationship to the chosen induction agent was obtained, allowing 10 min between each increment of drug addition, by which time steady state had been obtained.

Concentrations obtained in the bath were: thiopentone $9{\cdot}5 \times 10^{-6}$ to $6{\cdot}0 \times 10^{-4}$; propofol $9{\cdot}0 \times 10^{-6}$ to $1{\cdot}4 \times 10^{-4}$, or etomidate 10^{-8} to 10^{-4}M. Each preparation was exposed to one induction agent only and then discarded. Recordings were taken from at least six preparations for each induction agent at each concentration of verapamil or nifedipine. Time-matched controls were similarly exposed to induction agents in the absence of a calcium antagonist.

Contraction amplitude and frequency of the atria preparation was measured after each drug increment. The percentage depression of that amplitude was calculated by comparison with the trace at steady state equilibrium with verapamil, i.e. immediately before addition of the initial increment of induction agent. For each vein, the concentra-

Table 1. Effect of verapamil and nifedipine upon the −log EC10 (mean±s.e.m.) for thiopentone, propofol and etomidate with respect to depression of atrial frequency and tension of contraction.

Drug and dose (M)	Thiopentone		Propofol		Etomidate	
Control	Frequency	Tension	Frequency	Tension	Frequency	Tension
	4·01±0·06	4·23±0·22	4·67±0·19	5·03±0·24	4·33±0·16	6·52±0·27
Nifedipine						
10^{-10}	4·60±0·18	4·53±0·30	4·80±0·2	5·34±0·12	4·05±0·14	6·77±0·38
10^{-9}	4·38±0·11	4·43±0·25	5·12±0·10	5·05±0·13	3·90±0·33	5·92±0·40
10^{-8}	4·37±0·29	4·09±0·05	5·02±0·11	4·47±0·11	4·22±0·35	6·47±0·55
10^{-7}	4·10±0·13	4·78±0·13	4·66±0·12	5·00±0·07	4·50±0·36†	4·87±0·31†
Verapamil						
5×10^{-8}	4·10±0·24	4·14±0·35	5·26±0·13	5·18±0·27	3·5 ±0·35	7·17±0·18*
1×10^{-7}	4·11±0·19	4·87±0·04	4·85±0·23	5·46±0·31	5·12±0·25	7·76±0·23*
2×10^{-7}	4·30±0·1	4·54±0·17	5·21±0·13	5·30±0·38	5·20±0·69	7·38±0·40

* Significantly different from control value $P<0·05$.
† Mean of 3 preparations where depression occurred.

tion of agent causing 50% depression was calculated and expressed as the negative log EC50. It was not possible to obtain concentrations of the anaesthetics which caused 50% depression of either atrial rate or contraction tensions as the preparations ceased to beat before this point was reached. Thus the results from the atrial preparations were expressed as negative log EC10 values.

In a separate series of experiments using the portal veins alone, calcium concentration-response curves were obtained. After steady state equilibrium in standard Krebs-Henseleit solution, the preparations were washed and bathed in a calcium free Krebs-Henseleit solution. Maximal depression of the preparation was seen after 15 min, and a new steady state reached. Freshly prepared calcium chloride solution was then added cumulatively over 5 min intervals to obtain tissue bath concentrations of 0·25 to 8·0 mmol of calcium ions. Continuous recordings were made of the vein response and once complete the preparation was thoroughly washed and re-immersed in fresh calcium-free Krebs-Henseleit solution. The induction agent was then added to create a tissue bath concentration of thiopentone $9·5\times10^{-6}$ to $6·9\times10^{-4}$, propofol $9·0\times10^{-6}$ to $1·4\times10^{-4}$M and etomidate 10^{-8} to 10^{-4}M. Each preparation was exposed to one induction agent only, but calcium concentration-response relationships were performed for each concentration of agent in ascending order of strength on each preparation. Time-matched control preparations were exposed to the same sequence of calcium concentrations in the absence of anaesthetic induction agent, to assess temporal changes in preparation activity. Recordings for each induction agent were made on at least six preparations and six control sequences were performed.

Contraction amplitude was measured for each calcium concentration and concentration-response curves plotted. The maximum response was expressed as a percentage of the maximum response of the preparation before addition of the induction agent.

Student's *t*-test was then applied to assess significance. The three anaesthetics were prepared from commercially available ampoules. Control preparations were treated with vehicle alone. Verapamil and nifedipine solutions were prepared with distilled water. All nifedipine experiments were carried out under sodium lighting.

Results

All three intravenous anaesthetic agents possessed demonstrable negative chronotropic and inotropic effects on the isolated rat atria (Table 1).

Both thiopentone and propofol decreased contraction frequency and tension at similar concentrations (i.e. the negative log EC10 values were of the same order) whilst etomidate reduced the tension of contraction in lower concentrations (EC10 $3·0\times10^{-7}$M) than were necessary to decrease frequency (EC10 $4·6\times10^{-5}$M).

In general nifedipine had no significant effect on the response of the isolated atria to the three anaesthetic agents. Although in the presence of 10^{-7}M nifedipine, which reduced the contraction tension of the atria by 17·1±2·9%, etomidate failed to produce any further depression in 50% of preparations.

Verapamil did not alter the response of the atria to propofol and thiopentone or the frequency response to etomidate. However, the depression of the tension of contractions induced by etomidate was potentiated by verapamil (the EC10 for etomidate decreased from $3·0\times10^{-7}$ to 6·8, 1·7 and $4·2\times10^{-8}$M, respectively in the presence of the three concentrations of verapamil).

Table 2. Effect of verapamil and nifedipine upon the −log EC50 (mean±s.e.m.) of thiopentone, propofol and etomidate for depression of the spontaneous activity of the rat isolated portal vein.

Drug and dose (M) Control	Thiopentone	Propofol	Etomidate
	3·75±0·15	3·65±0·10	3·62±0·16
Nifedipine			
10^{-10}	3·95±0·10	4·42±0·18	4·53±0·24
10^{-9}	3·77±0·13	4·34±0·17	3·97±0·20
10^{-8}	3·51±0·11	3·60±0·21	4·28±0·31
10^{-7}	4·53±0·22	4·09±0·22	3·06±0·30
Verapamil			
5×10^{-8}	3·55±0·21	4·29±0·12	4·38±0·63
1×10^{-7}	4·23±0·18	4·18±0·23	5·98±0·32*
2×10^{-7}	3·86±0·17	3·97±0·27	7·03±0·53*
4×10^{-7}	4·14±0·18	4·96±0·41	5·86±0·49*

* Significantly different from control values $P<0·05$.

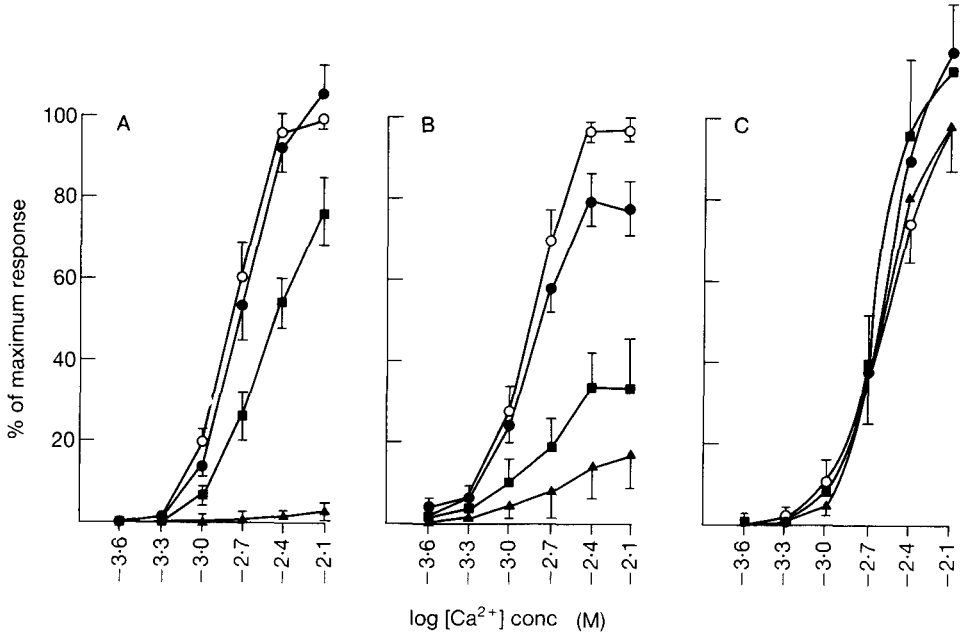

FIG. 1. Calcium concentration response relationships before the addition of anaesthetic agents (○) and after the addition of A thiopentone $3{\cdot}8 \times 10^{-5}$ (●), $1{\cdot}5 \times 10^{-4}$ (■) and $6{\cdot}0 \times 10^{-4}$M (▲), B propofol $9{\cdot}0 \times 10^{-8}$ (●), $3{\cdot}5 \times 10^{-5}$ (■) and $1{\cdot}4 \times 10^{-4}$M (▲), and C etomidate 10^{-9} (●) 10^{-6} (■) and 10^{-4}M (▲). Results are means ± s.e.m. of 6 preparations.

Verapamil alone caused some depression of the atrial preparations this being significant with the highest concentration used (−12·2 ± 6·5% frequency-13·8 ± 6·9% tensions); 4×10^{-7}M verapamil caused all preparations to arrest.

None of the vehicles for the anaesthetic agents had significant effects on the atria.

Negative EC50 values for each induction agent on the portal vein in the presence of each concentration of verapamil or nifedipine are shown in Table 2. The responses of the induction agents were compared with contraction amplitudes after the preparation had attained equilibrium with verapamil. There was a summation of action of verapamil and thiopentone and also between verapamil and propofol, since the negative log EC50 values for the induction agents remained unchanged in the presence of verapamil. Marked potentiation was seen when etomidate and verapamil were combined, since the negative log EC50 values of etomidate were significantly increased in the presence of verapamil. The EC50 for etomidate decreased from $2{\cdot}3 \times 10^{-4}$ to $9{\cdot}3 \times 10^{-8}$M in the presence of 2×10^{-7}M verapamil. The potentiation increased with increasing concentrations of verapamil until the highest concentration (4×10^{-7}M), where less potentiation was observed. This concentration depressed the portal vein by 42·8 ± 3·3% before addition of the etomidate; further changes were difficult to measure accurately using the recording system available. The negative log EC50 for etomidate was not altered in time-matched control preparations without verapamil. Potentiation of etomidate was not seen with nifedipine-treated preparations.

Calcium concentration-response curves are shown in Fig. 1. When results were compared with time-matched controls, etomidate was without significant effect on responses to calcium ion concentrations but thiopentone and propofol both produced a concentration-dependent reduction in the maximum response to calcium.

None of the drug vehicles used when administered alone had any effects on the preparation.

Discussion

All three induction agents caused some inhibition of atrial contraction and the rhythmic contractions of the portal vein.

In general, the responses of the tissues to propofol were similar to those to thiopentone. The depressant actions of both drugs on the atria and on portal vein summated with those of the two calcium entry blockers. In addition, both drugs reduced the response of the portal vein to calcium. The possibility that this latter action is common to all agents capable of causing general anaesthesia is negated by the observation that etomidate lacked this property even at concentrations which significantly reduced the spontaneous activity of the portal vein.

The potentiations of the action of etomidate by verapamil, but not by nifedipine, on the atrial preparation and, more dramatically, on the portal vein, suggest that the calcium antagonism produced by verapamil is not important for the interaction. Both etomidate (Benoit et al 1987) and verapamil (Nayler & Dillon 1986) can block sodium currents. However, tetrodotoxin, which also blocks sodium currents is without effect on the spontaneously action of the guinea-pig portal vein (Ito & Kuriyama 1971) suggesting that sodium currents do not contribute to the activity of that preparation. Earlier work by Johansson & Ljung (1967) indicates that the rat portal vein has similar properties.

Nevertheless, effects on sodium channels may be relevant to the interaction of the two drugs on the isolated atria.

There are many other ion channel receptor systems and enzyme systems affected by both etomidate and verapamil (Benoit et al 1987; Nayler & Dillon 1986) and more sophisticated techniques will be required to investigate the interaction further.

Heykants et al (1975) reported that 0·004 mM etomidate in rat brain was sufficient to produce general anaesthesia (negative log: 5·39). Thus the concentrations of etomidate necessary to inhibit the vein and atria in the presence of verapamil are comparable with the anaesthetic use of the drug, at least in the rat. Any extrapolation to the whole animal or to other species cannot be made.

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