INTERACTIONS BETWEEN CALCIUM ENTRY BLOCKERS AND VECURONIUM BROMIDE IN ANAESTHETIZED CATS

K. A. ANDERSON AND R. J. MARSHALL

Calcium antagonists (preferably called calcium entry blockers) are a structurally heterogeneous group of substances which share the common property of inhibiting the excitation-induced inward movement of calcium ions to cardiac and smooth muscle cells (Fleckenstein, 1977; Naylor and Poole-Wilson, 1981). Because excitation-contraction coupling in skeletal muscle is thought to depend mainly on intracellular stores of calcium, calcium entry blockers might not be expected to exert any significant effects on voluntary muscle contraction. However, recent studies have suggested that the calcium entry blocker, verapamil, can inhibit neuromuscular transmission in anaesthetized cats and dogs (Kraynack, Lawson and Gintautas, 1983; Lawson, Kraynack and Gintautas, 1983). In addition, a few preliminary reports have suggested that calcium entry blockers may potentiate the effects of some neuromuscular blocking agents both in isolated preparations (Bikhazi, Leung and Foldes, 1982; Kraynack, Lawson and Gintautas, 1982), and in anaesthetized rats (Bikhazi, Leung and Foldes, 1983) and rabbits (Durant, Nguyen and Katz, 1984). The purpose of the present study was to investigate, in chloralose-anaesthetized cats, any possible interaction between three calcium entry blocking agents (nifedipine, verapamil and bepridil) and the non-depolarizing neuromuscular blocking agent, vecuronium bromide, were investigated in chloralose-anaesthetized cats. In i.v. doses which caused decreases in arterial pressure, the calcium entry blockers did not affect indirectly-elicited twitches of tibialis muscle, but did potentiate the effects of vecuronium. No such potentiation of vecuronium was observed with the vasodilator drug, sodium nitroprusside. Experiments using close-arterial injections of acetylcholine suggested that the probable site of interaction is on the postsynaptic muscle membrane and probably involves blockade of calcium channels in skeletal muscle.

MATERIALS AND METHODS

Cats of either sex (weight range 1.8–3.0 kg) were used. Anaesthesia was induced initially with 3% halothane in oxygen to permit the administration of α-chloralose 80 mg kg⁻¹ i.v. After tracheal cannulation, the lungs were ventilated with room air by means of a Palmer pump (model 16/24) at a rate of 28 strokes min⁻¹ and a respiratory volume of 15 ml kg⁻¹ (later adjusted to maintain normal arterial blood pH). The tibialis anterior muscle was partially dissected free from surrounding tissue, and the tendon freed from its insertion to the calcaneous and attached to a Grass force displacement transducer (model FT03). A resting tension of 30 g was applied to the muscle. To ensure stability of the preparation, the leg was immobilized by placing a clamped pin through the patella and by clamping the lower portion of the leg. After the left sciatic nerve was crushed centrally, the peripheral end was stimulated in the popliteal space via bipolar electrodes using a Grass S88 stimulator and a frequency of 0.1 Hz, pulse duration 0.2 ms and a voltage of twice the strength required to evoke a maximal contraction of the muscle. The muscle and nerve were bathed in liquid paraffin. Arterial pressure was recorded from the left carotid artery through a polythene catheter (Bell and Howell pressure transducer). Heart rate was integrated from the arterial

K. A. ANDERSON, H.N.C. (BIOL.); R. J. MARSHALL, B.SC., PH.D.; Department of Pharmacology, Scientific Development Group, Organon Laboratories Ltd, Newhouse, Lanarkshire.
pressure trace and recorded using a Lectromed ratemeter. Tibialis twitch, arterial pressure and heart rate were recorded on a Lectromed M19 pen recorder. Drugs were injected to a jugular vein via a polythene catheter and washed in with physiological saline 2 ml. Throughout each experiment arterial blood samples (1 ml) were taken and analysed for $P_{O_2}$, $P_{CO_2}$ and pH (IL 1302 blood-gas analyser). Rectal temperature was measured using an Ellab TE3 thermometer and maintained (by heating lamps) at $37 \pm 0.5 ^\circ C$. Anaesthesia was maintained throughout the experiment using further bolus doses of $\alpha$-chloralose as necessary.

Two basic experimental programmes were used. The first was based on a previous observation that the cumulative administration of vecuronium produced consistent dose–response curves when repeated at hourly intervals over a prolonged period (McIndewar and Marshall, 1981). A cumulative dose–response curve to vecuronium was first constructed by injecting a bolus of 20 or 25 $\mu$g kg$^{-1}$ followed, after 3 min, by additional bolus doses of 5.0 or 7.5 $\mu$g kg$^{-1}$. Each increment was administered at the time of maximal blockade produced by the preceding dose until 80–90% neuromuscular blockade was achieved. At least two control cumulative dose–response curves were obtained (at hourly intervals to avoid any cumulation) to verify consistency of responses. Each dose–response curve was analysed by least-squared regression analysis and the dose producing 50% twitch block ($EC_{50}$) calculated.

Five minutes after the administration i.v. of bepridil 5 mg kg$^{-1}$ ($n = 4$, over 3 min), verapamil 0.5 mg kg$^{-1}$ ($n = 4$, over 3 min) or nifedipine 50 $\mu$g kg$^{-1}$ ($n = 3$, rapid injection), a cumulative dose–response to vecuronium was repeated. Sixty minutes after full recovery from neuromuscular blockade (some 75 min after administration of the calcium entry blocker) a further dose–response curve was obtained.

In the second series of investigations ($n = 5$), a dose of vecuronium was chosen which produced 80–90% blockade of twitches of the tibialis muscle. This dose was repeated at hourly intervals until consistent responses were obtained. Fifty minutes later, bepridil 5 mg kg$^{-1}$ was administered i.v. over 3 min and the dose of vecuronium repeated 5 min and again 75 min after the administration of bepridil. This regimen allowed at least 60 min between subsequent administrations of the neuromuscular blocking agent. Indices of neuromuscular blockade measured were:
(a) Degree of twitch blockade (%)
(b) Onset time (time from injection to maximum block)
(c) Recovery rate (time from 75% to 25% block)
(d) Duration (time from injection to recovery to 90% of pre-vecuronium twitch height).

In an attempt to elucidate the mechanisms involved in any interaction produced, the above regimen was repeated with the following modifications:
(A) An equivalent neuromuscular blocking dose of the depolarizing agent suxamethonium ($n = 4$) was used in place of vecuronium.
(B) Administration of an equivalent vasodilating dose of sodium nitroprusside ($n = 4$) was used in place of bepridil.

**Potentiation**

To gain some insight to the mechanisms underlying the potentiation, two preliminary studies were undertaken in which the effects of cumulative doses of vecuronium on both nerve-evoked twitches and contractions produced by close intra-arterial injection of acetylcholine were studied. These injections were given before and at the point of maximum neuromuscular blockade produced by vecuronium, in both the absence and presence of the calcium entry blocker, bepridil.

**Vehicles**

Bepridil was initially dissolved in polyethylene glycol and made up with distilled water to give a polyethylene glycol solution of 66%. Verapamil was dissolved in distilled water. Nifedipine was initially dissolved in ethanol then diluted with an equal volume of polyethylene glycol to give a nifedipine concentration of 440 $\mu$g ml$^{-1}$. Nifedipine solutions were protected from light throughout these experiments. Equivalent concentrations of these drug vehicles produced no significant effects on muscle twitch, arterial pressure or heart rate.

Statistical analysis of the results was performed using Student's paired $t$ test.

**RESULTS**

**Cardiovascular effects of the calcium entry blockers**

The doses used in the present study (bepridil 5.0 mg kg$^{-1}$, verapamil 0.5 mg kg$^{-1}$ and nifedipine 0.05 mg kg$^{-1}$) produced essentially similar and
transient decreases in arterial pressure (table I), an effect in keeping with their calcium entry blocking properties. All three drugs produced a decrease in heart rate, although this effect was sustained only in the case of bepridil (table I).

Effects of the calcium entry blockers on cumulative dose–response curves to vecuronium

In contrast to their marked effects on arterial pressure or heart rate, or both, bepridil, verapamil and nifedipine per se did not produce significant effects on indirectly-evoked twitches of the tibialis. Occasionally, slight (< 10%) and transient increases in twitch height were seen immediately on injection of each calcium entry blocker. However, all three agents potentiated significantly the neuromuscular blocking effects of vecuronium (table II) as assessed by a decrease in the EC_{50} value obtained by computer regression analysis.

The control data clearly show that the solvent control (66% propylene glycol) did not influence the EC_{50} values for vecuronium. The potentiating effects of verapamil and nifedipine were not apparent 75 min after administration, whereas the effects of bepridil were more sustained. Recovery time from vecuronium-induced blockade was not significantly modified by any of the calcium entry blockers. Analysis of the complete vecuronium dose–response curves showed that the magnitude of the leftwards shift varied from 1.3 (with verapamil and nifedipine) to 1.7 with bepridil. This degree of potentiation of vecuronium is similar to that previously reported with gaseous anaesthetic agents (McIndewar and Marshall, 1981).

Effects of bepridil on neuromuscular blocking actions of bolus injections of vecuronium or suxamethonium

In order to study possible effects of the calcium entry blockers on the speed of onset of vecuronium...
(which could not be assessed from cumulative dose–response curve studies), we studied the influence of bepridil on a 90% neuromuscular blocking bolus dose of vecuronium. The results are summarized in figure 1. In the five experiments the bolus dose of vecuronium used ranged from 25.0 to 57.5 μg kg\(^{-1}\). As can be seen from this figure, consistent responses (with respect to magnitude of blockade, onset time and recovery rate) were obtained with the control bolus injections of vecuronium administered 60 min apart. When the administration of vecuronium was repeated 5 and 75 min after the administration of bepridil 5 mg kg\(^{-1}\), there was an increase in the magnitude of blockade (to 100% in all experiments) accompanied by a decrease in onset time. Recovery rate of vecuronium, which is largely independent of degree of blockade, tended to increase after the administration of bepridil, but the changes were not significant. When vecuronium was replaced by equiactive bolus administration of suxamethonium (50–80 μg kg\(^{-1}\)), no such modification by bepridil was apparent (fig. 2). One interesting observation made in these last experiments was that muscle fasciculations, which are characteristic of the depolarization-induced blocking action of suxamethonium, were greatly diminished after the administration of bepridil.

**Effects of sodium nitroprusside on arterial pressure and heart rate, and on the neuromuscular blocking effects of vecuronium**

The dose chosen (0.015 mg kg\(^{-1}\)) of the peripheral vasodilator, sodium nitroprusside, produced a transient decrease in arterial pressure similar to that produced by the three calcium entry blockers (table 1). However, in contrast to the latter agents, the vasodepressor actions of sodium nitroprusside were accompanied by a small but significant tachycardia. This vasodilator dose of
sodium nitroprusside did not modify the magnitude or time course of the neuromuscular blocking effects of bolus administration of vecuronium (fig. 3).

Modification by bepridil of effects of vecuronium on acetylcholine-induced muscle contractions

In two cats, we compared the neuromuscular blocking effects of cumulative doses of vecuronium on both nerve-evoked twitches and contractions of tibialis muscle produced by close-arterial injection of acetylcholine. The results of one of these experiments are shown in figure 4. When administered close-arterially, acetylcholine 5 μg produced consistent contractions of the tibialis muscle. When repeated at the height of vecuronium-induced blockade of nerve-evoked twitches (81 and 76%, respectively, in the two control runs) responses to acetylcholine were also consistently reduced (by 93 and 90%, respectively). When twitches had just returned to control values, responses to acetylcholine were still somewhat depressed (by 76 and 73%, respectively).

Bepridil 5 mg kg\(^{-1}\) i.v. did not itself modify the acetylcholine-induced contraction (fig. 4). However, as observed previously, bepridil potentiated the blocking effects of vecuronium on indirectly-evoked twitches so that 83% blockade was produced by a cumulative dose of 32.5 μg kg\(^{-1}\) (compared with 47.5 μg kg\(^{-1}\) in both control runs). Blockade of the acetylcholine response produced by vecuronium 32.5 μg kg\(^{-1}\) (i.e. 92%) was similar to that obtained with vecuronium 47.5 μg kg\(^{-1}\) in the absence of bepridil. Similar results were obtained in the other cat, in which the cumulative dose of vecuronium causing comparable blockade of both twitches (71 and 79%) and acetylcholine contractions (63 and 65%) was reduced from 62.5 μg kg\(^{-1}\) in the absence of bepridil to 47.5 μg kg\(^{-1}\) 5 min after administration of the calcium entry blocker. Thus, bepridil would appear to potentiate equally the blocking effects of

![Figure 4](image-url)
vecuronium on muscle contractions, whether produced by neurally-released or close-arterially injected acetylcholine.

**DISCUSSION**

Although it is generally held that external Ca\(^{2+}\) is not necessary for contraction of mammalian skeletal muscle (Luttgau and Spiecker, 1979), the demonstration of inward Ca\(^{2+}\) currents which can be abolished by calcium entry blocking drugs in these muscles (Gonzalez-Serratos et al., 1982) prompted us to re-examine the effects of some calcium entry blocking agents on neuromuscular transmission, and on the effects of neuromuscular blocking drugs in fast-contracting tibialis muscle of the chloralose-anaesthetized cat. The doses of the calcium entry blockers were chosen to produce similar decreases in arterial pressure, an effect caused by their blocking action on calcium channels in peripheral vascular smooth muscle (Stone et al., 1980). In keeping with their different relative selectivities for calcium channels in cardiac muscle (Henry, 1980; Kawada, Satoh and Taira, 1983), only bepridil produced a marked and sustained bradycardia. In contrast to their effects on calcium channels in vascular smooth or cardiac muscle, or both, none of the calcium entry blockers significantly modified neuromuscular transmission per se. This finding is in agreement with previous observations in isolated preparations (Gonzalez-Serratos et al., 1982; Bikhazi, Leung and Foldes, 1982; Fairhurst et al., 1983) and in small anaesthetized mammals (Bikhazi, Leung and Foldes, 1983; Durant, Nguyen and Katz, 1984). In contrast, another group of workers has shown that verapamil can reduce indirectly-evoked twitches of skeletal muscle both in isolated amphibian preparations (Kraynack et al., 1983) and in anaesthetized cats (Kraynack, Lawson and Gintautas, 1983) and dogs (Lawson, Kraynack and Gintautas, 1983). It is interesting to note that, in these last studies, a stimulation frequency of 1 Hz was used (i.e. 10 times that used in our study) which would be expected, perhaps, to decrease the margin of safety of neuromuscular transmission.

In spite of their minimal effects on neuromuscular transmission, all three calcium entry blockers significantly potentiated the neuromuscular blocking effects of cumulatively administered vecuronium. Similar interactions between neuromuscular blockers and calcium entry blockers have recently been reported in both isolated rat diaphragm preparations (Bikhazi, Leung and Foldes, 1982), and in anaesthetized rabbits (Durant, Nguyen and Katz, 1984) and rats (Bikhazi, Leung and Foldes, 1983). In the present studies, the calcium entry blockers shifted the dose-response curves to vecuronium in a parallel manner and by a degree similar to that previously reported for inhalation anaesthetics (McIndewar and Marshall, 1981).

We have also shown that bepridil potentiates the magnitude of blockade (and shortens the onset time) produced by bolus injections of vecuronium. Somewhat surprisingly, no such effects of bepridil were observed when the depolarizing blocker, suxamethonium, was used in place of vecuronium, although bepridil did appear to reduce the muscle fasciculations produced by the depolarizing blocker. The ability of bepridil to potentiate the blockade and to decrease markedly the onset time of vecuronium would seem unlikely to be the result of an increased delivery of blood to the muscles, since another vasodilator drug, sodium nitroprusside, did not modify the magnitude or time-course of the neuromuscular blocking actions of vecuronium. It is interesting to note that the mechanism of the vasodilator actions of sodium nitroprusside does not involve inhibition of membrane calcium channels (Zsoter, Henein and Wolchinsky, 1977).

Although the present studies were not designed primarily to investigate the mechanisms underlying these interactions, several possibilities seem more likely than others. The preliminary observation in two animals, that bepridil potentiated vecuronium-induced blockade of both nerve-evoked and injected acetylcholine-evoked contractions to a similar degree, suggests to us that a postjunctional interaction seems more likely than an effect on nerve conduction or on acetylcholine release. In addition, in the dose used in this study, nifedipine would not be expected to modify nerve conduction (Hay and Wadsworth, 1982). The inability of nifedipine readily to penetrate either smooth or cardiac muscle cells (Pang and Sperelakis, 1983) suggests that a direct action on contractile proteins in skeletal muscle seems unlikely also. Nifedipine, verapamil and bepridil differ markedly in potency regarding their ability to inhibit calcium channels in smooth muscle (Pang and Sperelakis, 1983) suggests that a direct action on contractile proteins in skeletal muscle seems unlikely also. Nifedipine, verapamil and bepridil differ markedly in potency regarding their ability to inhibit calcium channels in smooth muscle (Kawada, Satoh and Taira, 1983). The present observation that equiactive vasodilator doses of these three calcium entry blockers produce similar potentiation of the neuromuscular blocking actions of vecuronium suggests that blockade of calcium channels in
skeletal muscle may be involved in the observed interactions. The recent demonstrations, using either voltage clamp (Chiriandini and Stefani, 1983) or ligand binding studies (Glossman, Ferry and Boschek, 1983), of calcium channels in mammalian skeletal muscle sarcolemma add some credence to this hypothesis. We cannot, however, rule out an additional effect of verapamil or bepridil on fast sodium channels, since these two drugs are not as selective as nifedipine for calcium channels (Labrid et al., 1979; Hay and Wadsworth, 1982). Whatever the actual mechanism(s) involved, the present data and previous results of others, suggest that anaesthetists should be aware of such potential interactions in patients receiving long-term therapy with calcium entry blocking agents. Indeed, a case describing potentiation of vecuronium in a patient with renal failure receiving verapamil has been reported recently (van Poorten et al., 1984).

REFERENCES


