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Functional interactions of calcium-antagonists in K^+-depolarized smooth muscle

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1 The functional significance of the interaction of certain calcium-antagonists with nimodipine was examined so that this might be related to binding studies. To this end, the relaxant effects of nimodipine (1–100 nM) on Ca^{2+} (10 mM) -induced contractions of K^+-depolarized taenia preparations from the guinea-pig caecum were compared in the presence of nifedipine and cinnarizine (which competitively displace [^{3}H]-nimodipine with high and low affinities respectively), diltiazem (which increases binding), verapamil (which allosterically reduces binding) and W-7, a calmodulin antagonist.

2 The relaxant effects of nimodipine were similar in the presence of nifedipine, diltiazem and cinnarizine, but were slightly attenuated in the presence of verapamil and W-7.

3 These findings can be reconciled with the differentiation of calcium antagonists evident from [^{3}H]-nimodipine binding studies, but indicate that the functional consequences of allosteric interactions disclosed in such studies are small. Drugs which bind to calcium channels and drugs which bind to calmodulin did not potentiate each other.

Introduction

Displacement of [^{3}H]-nimodipine binding has been claimed to differentiate four subgroups of calcium-antagonists (Glossmann, Ferry, Lübbecke, Mewes & Hofmann, 1982; Ferry & Glossmann, 1982). Two of these subgroups, the dihydropyridines and the diphenylalkylamines (e.g. cinnarizine, flunarizine, fendiline), displace [^{3}H]-nimodipine binding with high and low affinity respectively and in each case display single site displacement isotherms corresponding to the law of mass action. In contrast, the two other subgroups display allosteric interactions; (−)−verapamil and methoxyverapamil cause biphasic inhibition of binding whereas diltiazem increases binding by slowing dissociation of [^{3}H]-nimodipine from its receptor. Similar differences have been reported when [^{3}H]-nitrendipine is used as the ligand (De Pover, Matlib, Lee, Dubé, Grupp, Grupp & Schwartz, 1982; Yamamura, Schoemaker, Boles & Roeske, 1982; Murphy, Gould, Largent & Snyder, 1983). In contrast to these ligand binding data, functional studies have only differentiated two (Fleckenstein, 1981) or three (Spedding, 1981; 1982a, b, c; 1983) sub-types. Neither of these functional classifications differentiated the effects of diltiazem from those of verapamil.

The [^{3}H]-dihydropyridine binding site is claimed to be the calcium channel, although certain discrepancies exist. Dihydropyridines exert considerable selectivity for vascular smooth muscle compared with the myocardium, but selectivity is slight in binding studies (Williams & Tremble, 1982). Furthermore, the brain has many dihydropyridine binding sites (Glossmann *et al.*, 1982; Murphy, Gould & Snyder, 1982) but dihydropyridines do not have marked central effects. I have therefore tested for functional interactions between representatives of the proposed different classes of calcium-antagonist and the most selective dihydropyridine currently available, nimodipine (Bellemann, Ferry, Lübbecke & Glossmann, 1982). The relaxant effects of nimodipine were compared on K^+-depolarized taenia preparations from the guinea-pig caecum pretreated with approximately equivalent blocking doses of calcium antagonists, the effects of which were surmounted with a high concentration of Ca^{2+} (10 mM). Thus, the sensitivity of concentration-response curves to nimodipine in the presence of a pre-equilibrated calcium-antagonist may reflect nimodipine binding. Furthermore, should nimodipine and the other drugs act at different sites the effects of nimodipine would be expected to be potentiated if the interaction is equivalent to drugs acting at two different receptors. For this reason the effects of nimodipine were also assessed in

the presence of N-(6-aminohexyl)-5-chloro-1 naphthalenesulphonamide (W-7), a drug that binds to calmodulin and the contractile proteins (Hidaka, Asano, Iwadare, Matsumoto, Totsuka & Aoki, 1978) and inhibits contractility in the taenia while increasing cellular Ca^{2+} content (Karaki, Murakami, Nakagawa, Ozaki & Urakawa, 1982). The taenia preparation was chosen as it is very sensitive to all the antagonists and responsiveness to Ca^{2+} does not change with time (Spedding, 1982a).

Methods

Taenia preparations from the caecum of male guinea-pigs (150–250 g) were set up in 10 ml isolated organ baths containing K^+-depolarizing Tyrode solution (composition mM: NaCl 97, KCl 40, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5). The Tyrode solution was maintained at 35 ± 0.5°C and gassed with 95% O_2:5% CO_2. Contractile responses were recorded using isotonic transducers (1 g load) and Rikadenki flat bed recorders. After 30 min a cumulative concentration-response curve to Ca^{2+} (0.1–10 mM; Spedding, 1982a) was obtained. Following washout of the Ca^{2+} and relaxation of the tissues, a representative calcium-antagonist from each of the proposed four antagonist subgroups (Ferry & Glossmann, 1982) was incubated with the tissue for 20 min in concentrations chosen to give roughly equivalent antagonistic effects. The concentration-response curve to Ca^{2+} was then repeated. The actual concentrations and the dose-ratios (range of the s.e.mean) were: nifedipine 3 nM, 15.1 (10.5–21.4) $n=7$; diltiazem 1 μM, 38.0 (26.3–53.7) $n=8$; verapamil 0.2 μM, 19.5 (12.0–31.6) $n=9$; cinnarizine 1 μM, 10.0 (7.1–14.1) $n=7$; W-7 200 μM 6.5 (3.6–11.5). In each case, the highest concentration of Ca^{2+} used (10 mM) caused 60–85% of the maximum response and this contraction was stable indicating that equilibrium conditions pertained for the antagonists. A cumulative concentration-response curve to the relaxant effects of nimodipine was then obtained in the presence of Ca^{2+} (10 mM) and the equilibrated calcium-antagonist.

The following drugs were used: diltiazem hydrochloride and W-7 (Synthelabo), cinnarizine tartrate (Janssen Pharmaceutica), (±)-verapamil hydrochloride (Knoll), nifedipine and nimodipine (Bayer AG). Solutions of nifedipine and nimodipine were dissolved in ethanol (0.1 mM) and diluted in distilled water. The experiments were performed in a darkened room and all organ baths and dihydropyridine solutions were protected from light.

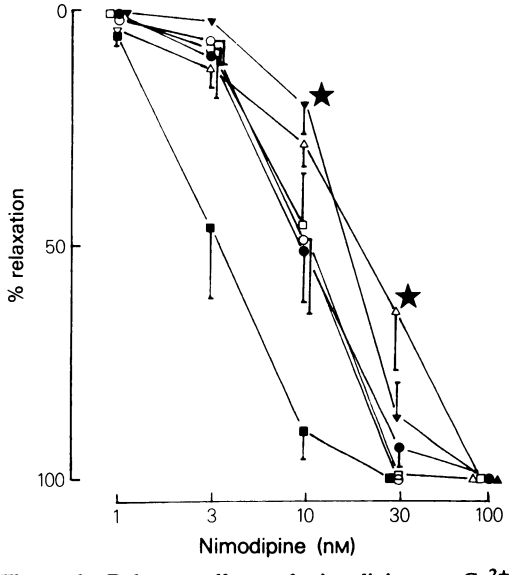

Figure 1 Relaxant effects of nimodipine on Ca^{2+} (10 mM)-induced contractions in K^+-depolarized taenia preparations from the guinea-pig caecum. The nimodipine concentration-response curves were obtained in the presence of nifedipine (3 nM, ●, $n=7$), diltiazem (1 μM, □, $n=8$), cinnarizine (1 μM, ○, $n=8$), W7 (200 μM, △, $n=6$) or verapamil (0.2 μM, ▼, $n=9$). *$P<0.05$ compared with relaxant effects in the presence of cinnarizine, nifedipine or diltiazem (Mann-Whitney, U test). Vertical bars represent s.e.mean. The entire verapamil and W-7 curves were different ($P<0.01$) from the other antagonists when assessed by analysis of variance, although the variances were not homogeneous (Bartlett's test). The effects of nimodipine on Ca^{2+} (1 mM)-induced contractions in preparations not previously exposed to calcium-antagonists (■, $n=6$) are also shown.

Results

Nimodipine (1–100 nM) relaxed Ca^{2+} (1 mM)-induced contractions (onset < 5 min, half maximal < 12 min, optimal < 60 min, Figure 1). In the experiments where the preparations had been pretreated with the various calcium antagonists (nifedipine 3 nM, verapamil 0.2 μM, diltiazem 1 μM, cinnarizine 1 μM, W7 200 μM) and recontracted with Ca^{2+} (10 mM), the nimodipine concentration-response curves were displaced to the right. However, the sensitivity to nimodipine (1–100 nM) was equivalent in the presence of nifedipine, cinnarizine or diltiazem (Figure 1). The preparations which had been incubated with verapamil and W-7 were significantly less sensitive to nimodipine (Figure 1).

Discussion

With the exception of a small displacement with verapamil and W-7, there were no functional differences between the effects of the different classes of antagonists on sensitivity to nimodipine. The most marked difference was between the preparations pretreated with calcium antagonists and the control tissues. This was to be expected because the two situations are not directly comparable. In the pretreated preparations there will be fewer unblocked channels for nimodipine to interact with, and these channels will have a higher Ca^{2+} flux due to the elevated extracellular Ca^{2+} concentration (10 mM compared with 1 mM) used to recontract the tissue. Nevertheless, the displacement to the right of the curves in the pretreated tissues does mean that the effects of nimodipine are reduced rather than potentiated by the other calcium antagonists.

Thus, diltiazem did not enhance the nimodipine-induced relaxations compared with the effects of nifedipine and cinnarizine. This finding does not necessarily mean that diltiazem did not increase nimodipine binding because, unlike verapamil, diltiazem slows dissociation of nimodipine without affecting affinity (Ferry & Glossmann, 1982). Diltiazem will only increase dihydropyridine binding while it is blocking a channel and when diltiazem dissociates from the channel the effect will be lost and this interaction will therefore have little functional consequences in smooth muscle.

The relaxant effects of nimodipine were also similar whether the preparations were pretreated with nifedipine or cinnarizine which is compatible with the binding data; the high and low affinities of these agents are reflected in the concentrations used to cause the initial antagonist effects (3 nM and 1 μM). These findings might be taken to indicate that the functional site of action of both drugs is the calcium channel, as defined by dihydropyridine binding, but certain reservations must be made. Calmodulin antagonists are not distinguishable from diphenylalkylamines in binding studies (Kaufman & Conery, 1983) and as calmodulin is not the dihydropyridine binding site (Norman, Borsotto, Fosset, Lazdunski & Ellory, 1983) then the interaction of these agents with the channel will be due to their non-specific lipophilic properties, a characteristic of all these compounds which parallels binding to calmodulin (Landry, Amellal & Ruckstuhl, 1981). Thus, any interaction with the calcium channel will be paralleled by lipophilic interactions at other sites, such as the contractile proteins (Spedding, 1982c). In this respect, the failure of W-7 to increase the sensitivity of nimodipine indicates that the functional interactions between drugs acting at the channel and at the contractile proteins are relatively small, at least under the present experimental conditions.

The sensitivity to nimodipine was reduced by verapamil, compared with nifedipine, diltiazem and cinnarizine. This may reflect the allosteric interaction reported between verapamil and nimodipine binding, but could also have been due to an additional effect of verapamil, such as displacement of extracellular Ca^{2+} at the cell surface (Pang & Sperelakis, 1982).

In conclusion, these experiments show that the functional consequences of interactions between calcium antagonists are small in smooth muscle. Nevertheless, there are many functional dissimilarities between the effects of the compounds *per se* (Fleckenstein, 1981; Spedding, 1981, 1982a, b, c) and subclassification of the drugs on these grounds is in broad agreement with the subclassification based on dihydropyridine binding studies (Glossmann *et al.*, 1982; Ferry & Glossmann, 1982).

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