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Effects of calcium channel antagonists on action potential conduction and transmitter release in the guinea-pig vas deferens

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1 The effects of the Ca^{2+} channel antagonists amlodipine, cobalt, diltiazem, nifedipine and verapamil and the local anaesthetic lignocaine were investigated on action potential conduction in and on evoked transmitter release from sympathetic nerves in the guinea-pig isolated vas deferens. Transmitter release was investigated by measurement of (a) evoked (trains of pulses at 1 and 2 Hz, 0.1–0.5 ms supramaximal voltage) excitatory junction potentials (e.j.ps) using microelectrodes; tension was recorded simultaneously; (b) tritium (^3H) overflow from vasa preincubated (37°C, 30 min) in Krebs solution containing either [^3H]-noradrenaline (NA, 25 μCi ml^{-1}, 2×10^{-6} M NA) or [^3H]-adenosine (50 μCi ml^{-1}, 1×10^{-6} M adenosine).

2 Amlodipine ($0.5-2 \times 10^{-4}$ M), verapamil ($0.5-2 \times 10^{-4}$ M), diltiazem ($1-8 \times 10^{-4}$ M), lignocaine ($0.1-2 \times 10^{-3}$ M) and cobalt ($2-6 \times 10^{-2}$ M) in descending order of potency, but not nifedipine ($1-5 \times 10^{-3}$ M), increased the latency and inhibited, then abolished, the amplitude and number of action potentials in a concentration-dependent manner.

3 Amlodipine ($0.5-1 \times 10^{-4}$ M), verapamil ($1-2 \times 10^{-4}$ M), diltiazem ($1-5 \times 10^{-4}$ M) and cobalt (1×10^{-3} M), in descending order of potency, but not nifedipine (5×10^{-4} M), inhibited then abolished evoked e.j.ps in a concentration-dependent manner. Cobalt inhibited e.j.ps at a lower concentration than that ($2-6 \times 10^{-2}$ M) required to block action potential conduction.

4 In unstimulated tissues, the resting ^3H overflow following preincubation with [^3H]-NA consisted largely of 4-hydroxy 3-methoxymandelic acid (VMA), 4-hydroxy 3-methoxy phenylglycol (MOPEG), 3,4 dihydroxyphenylglycol (DOPEG) and NA; stimulated tissues (300 pulses at 20 Hz, 0.5 ms supramaximal voltage) released mainly NA. Verapamil ($0.1-1 \times 10^{-4}$ M), amlodipine ($0.05-1 \times 10^{-4}$ M) and nifedipine ($1-5 \times 10^{-4}$ M), but not cobalt (2×10^{-3} M), increased, significantly, the resting overflow of ^3H comprising mainly DOPEG. Cobalt (2×10^{-3} M) inhibited, significantly, the stimulation-evoked overflow of ^3H.

5 Verapamil (1×10^{-4} M) had little effect on the resting overflow of ^3H following preincubation with [^3H]-adenosine. Diltiazem (5×10^{-4} M) and cobalt (2×10^{-3} M) both inhibited evoked ^3H overflow. Nifedipine (5×10^{-4} M) was ineffective.

6 The effectiveness of Ca^{2+} channel antagonists at pre- and postjunctional sites differ; the results are discussed in terms of the selectivity of these drugs for each site and their differential effects on transmitter release.

Introduction

During the past few years, major advances in our understanding of the process by which transmitters are released from sympathetic nerves have been made (see Cunnane, 1984). For example, transmitter release from varicosities occurs intermittently following stimulation of the parent axon. The nature of the transmitter released has also been re-examined and there is evidence that in both vascular (Burnstock & Sneddon, 1984; Allcorn *et al.*, 1985) and non-vascular smooth muscle (Sneddon & Westfall, 1984) noradrenaline (NA) and an adenine nucleotide, probably adenosine triphosphate (ATP), may be released as co-transmitters from the same nerve ending. In view of these findings, it seemed of interest to re-examine

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agents known to block Ca^{2+} entry to determine their effects on those ionic channels which control transmitter release from nerve terminals.

The ability of calcium (Ca^{2+}) channel antagonists to inhibit Ca^{2+} influx, postjunctionally, into both cardiac and smooth muscle and the use of these drugs in the treatment of hypertension and angina are already well known (Fleckenstein, 1983). Ca^{2+} channel antagonists also increase or decrease transmitter release, effects which vary with different compounds and with the doses employed. Thus Ca^{2+} channel antagonists, albeit in high concentrations (1×10^{-5} M and greater), inhibit the release of transmitter evoked by nerve action potentials or by potassium (K^+) depolarization, as measured spectrofluorimetrically in the rabbit isolated heart (Göthert *et al.*, 1979) and radiochemically in the guinea-pig portal vein (O'Connor, 1982). On the other hand, verapamil and nifedipine in low ($3-5 \times 10^{-6}$ M and $0.3-3 \times 10^{-6}$ M respectively) though not at higher ($1-3 \times 10^{-4}$ M and 1×10^{-5} M, respectively) concentrations, increase the amplitude of inhibitory junction potentials (i.j.ps) in the guinea-pig ileum (Bornstein *et al.*, 1985). Indeed these higher concentrations reduced the amplitude of the i.j.ps in this tissue.

The ability to manipulate, selectively, the prejunctional release process would provide an important control of transmission. Accordingly, the effects of a number of Ca^{2+} channel antagonists have now been investigated to determine (1) their effects on transmitter release from sympathetic nerves of the guinea-pig vas deferens, (2) whether, in view of the release of two transmitters following nerve stimulation (Sneddon & Westfall, 1984), both NA and ATP liberation is affected in parallel and (3) if release is affected, does it occur as a consequence of interference with nerve action potential conduction or with Ca^{2+} entry into the invaded varicosity? The results are discussed in relation to current views of sympathetic neuroeffector transmission. Preliminary accounts have been communicated already to the British Pharmacological Society (Beattie *et al.*, 1984; 1985).

Methods

General

Male Duncan-Hartley guinea-pigs (300–1000 g) were killed by cervical dislocation and bled. One vas deferens with attached hypogastric nerve branches and connective tissue was removed and pinned to the Sylgard base of a horizontal organ bath (4 ml) bathed with Krebs solution of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, KCl 4.7, $CaCl_2$ 2.7, $MgCl_2$ 1.3, glucose 11.0. This bathing medium was maintained at 37 ± 0.5°C, and gassed with a 95% O_2 and 5% CO_2 mixture to pH 7.4. The vas which possessed no inherent tone of its own, was subjected to a tension of 1 g which was maintained throughout the experiment. Indirect stimulation (single pulses or trains of pulses at 1, 5, 10 and 20 Hz, 0.05–0.5 ms pulse width and supramaximal voltage) was carried out via a pair of Ag/AgCl ring electrodes placed around the postganglionic hypogastric nerves, which run alongside the prostatic end of the tissue. The electrodes were connected to a square pulse generator (Devices Mk III) and Digitimer (Devices). Drugs were either perfused in the Krebs solution or added directly to the organ bath.

(a) *Action potential conduction in the hypogastric nerves*

The hypogastric nerves, which run alongside the vas deferens from the prostatic to the epididymal end, branch along their course into smaller bundles. These nerves are often referred to as the vas deferens nerves (Cunnane & Stjärne, 1984). Following pinning of the tissue to the base of the organ bath (4 ml), one of these nerve bundles (30–100 μm in diameter) was cut, under microscopic control, near to its point of entry into the epididymal end of the tissue. This bundle was cut and the central end sucked into the tip of a glass microelectrode filled with Krebs solution. Into this tip was inserted an Ag/AgCl wire to allow electrical events in the nerve bundle to be measured. An Ag/AgCl pellet, located in the organ bath, served as the indifferent electrode. Suction was applied using a syringe (1 ml) filled with liquid paraffin and connected to the microelectrode by fine polythene tubing (1.5 mm o.d.). Pressure in the microelectrode was controlled by rotating the plunger of the syringe. Electrical activity was amplified (Neurolog NL 103), filtered (Neurolog NL 115) and displayed on a storage oscilloscope (Tektronix) from which signals were recorded and stored on an FM tape recorder (Racal 4DS).

(b) *Transmitter release*

The effects of Ca^{2+} channel antagonists on transmitter release were measured in two ways: (1) by conventional intracellular measurement of evoked (trains of pulses at 1 and 2 Hz, 0.1–0.5 ms pulse width, supramaximal voltage) excitatory junction potentials (e.j.ps) using microelectrodes. The mechanical response of the vas was measured, simultaneously, by connecting the prostatic end to a force-displacement transducer (Grass FT03C). Electrical activity was measured by capillary glass microelectrodes (resistance 20–49 MΩ) filled with 3 M KCl; signals were passed via a unity gain high impedance amplifier (WP Instruments, Model 4A) to a storage oscilloscope and u.v. recorder (S.E. Oscillograph 3006) and stored on tape (Racal 4DS). Results are expressed as means

± s.e.mean of a number (n) of observations. (2) By measurement of tritium (^3H) overflow from vasa preincubated (37°C, 30 min) in Krebs solution containing either (a) [^3H]-noradrenaline ([^3H]-NA, 25 $\mu Ci ml^{-1}$, 2×10^{-6} M NA) or (b) [^3H]-adenosine (50 $\mu Ci ml^{-1}$, 1×10^{-6} M adenosine), since both NA and adenosine triphosphate (ATP) are purported to have a transmitter function in this tissue (Sneddon & Westfall, 1984). After rinsing with Krebs solution, tissues were connected to a force-displacement transducer (Grass FT03C) under 1 g tension and mounted vertically inside a heated water jacket (37°C) to allow superfusion with Krebs solution.

Tissues preincubated with [^3H]-NA were superfused (1 ml min^{-1}, 37°C) with Krebs containing ascorbic acid (1.1×10^{-4} M) to inhibit catecholamine oxidation, desipramine (6×10^{-7}M) and normetanephrine (1×10^{-5} M) to inhibit neuronal and extraneuronal catecholamine uptake respectively and atropine (2.6×10^{-6}M) to block any cholinergic influence on the release process. Under these conditions, approximately 75% of ^3H released was unmetabolized [^3H]-NA (see results). Following two initial stimulation periods (20 pulses at 10 Hz, 0.5 ms pulse width, supramaximal voltage) to settle the tissue, field stimulation (60 pulses at 1, 5, 10 and 20 Hz, 0.5 ms, supramaximal voltage) was carried out before and during superfusion with the Ca^{2+} channel antagonists. The ^3H in the superfusate was separated into NA and its principal metabolites (Graefe *et al.*, 1973), i.e. 3,4-dihydroxyphenylglycol (DOPEG), 4-hydroxy,3-methoxymandelic acid (VMA) and 4-hydroxy,3-methoxyphenylglycol (MOPEG), 3,4-dihydroxymandelic acid (DOMA) and normetanephrine (NMN), by use of activated alumina (Crout, 1961) and Dowex 50WX4 (200–400 mesh) chromatography columns.

Tissues preincubated with [^3H]-adenosine were superfused with a hypertonic Krebs solution containing sucrose (12.5%), to minimize muscle contractions, α,β-methylene adenosine triphosphate (α,β-meATP) and prazosin (both 5×10^{-6} M) to inhibit, respectively, the effects of stimulation of non-adrenergic, non-cholinergic (NANC) and adrenergic nerves. One vas from each animal was superfused with the Ca^{2+} channel antagonists under investigation while the contralateral tissue served as a control. Both were stimulated simultaneously (400 and 2000 pulses at 20 Hz, 0.5 ms pulse width, supramaximal voltage).

Two-minute samples of the superfusate were collected and ^3H measured by liquid scintillation counting (Packard). At the end of each experiment, tissues were dissolved in potassium hydroxide (0.5 M) and the residual content of ^3H counted. The efficiency of counting was approximately 30%. Results (corrected to 100% efficiency) were expressed as disintegrations per minute (d.p.m.) or as the fractional release of ^3H, i.e. the overflow evoked by stimulation as a fraction (± s.e.mean) of the total ^3H in the tissue at that time (Alberts *et al.*, 1981).

Drugs

The following drugs were used; with the exception of TTX which was used as the base, concentrations refer to the salts. Except where otherwise stated, drugs were dissolved in 0.9% w/v NaCl solution (saline): 2,3,8-[^3H]-adenosine (Amersham International, 40–60 Ci $mmol^{-1}$, 97% pure, as commercially supplied), amlodipine maleate (Pfizer), (−)-ascorbic acid (Koch-Light), atropine sulphate (Sigma), choline chloride (Hopkin & Williams), cobaltous chloride (Koch-Light), desipramine hydrochloride (Ciba), (±)-3,4-dihydroxymandelic acid (Sigma), (±)-3,4-dihydroxyphenylglycol (Sigma), diltiazem hydrochloride (Sigma), 6-hydroxydopamine hydrobromide (Sigma), (±)-4-hydroxy,3-methoxymandelic acid, lignocaine (Sigma), α,β-methylene adenosine triphosphate (Sigma), nifedipine (Pfizer), (−)-7,8-[^3H]-noradrenaline (Amersham International, 8–14 Ci $mmol^{-1}$, 98% pure, as commercially supplied), (−)-noradrenaline bitartrate (Koch-Light), (±)-normetanephrine hydrochloride (Sigma), (±)-prazosin hydrochloride (Pfizer), sucrose (Formachem), tetrodotoxin (TTX, Boehringer), tris (hydroxymethyl) aminomethane hydrochloride (Sigma), verapamil hydrochloride (Sigma).

Nifedipine was dissolved under Na illumination in the minimum amount of cremophor EL (Sigma) and diluted in Krebs solution. Cremophor itself was inactive. Cobalt was prepared in a modified Krebs solution (Tris buffer 2.5×10^{-3} M and choline chloride 23.6×10^{-3} M replacing NaH_2PO_4 and $NaHCO_3$, respectively) to prevent precipitation of insoluble cobalt salts. Controls contained only this modified Krebs solution.

6-Hydroxydopamine (6-OHDA) was dissolved by sonication in saline, containing ascorbic acid (5.7×10^{-3} M), kept on ice and bubbled with O_2-free N_2 for at least 30 min before use. The drug was given i.p. in doses of 150 mg kg^{-1} on day 1 and 250 mg kg^{-1} on day 2; experiments were carried out on day 3. One vas from each of the 6-OHDA-treated animals was subjected to a modification of the Falck histochemical procedure (Gillespie & Kirpekar, 1966) and examined microscopically for the extent of the degeneration of adrenergic nerves. Sections (6 μm thick) were cut and mounted in a drop of liquid paraffin. The fluorescing specimens were viewed and photographed with a Carl Zeiss ACM photomicroscope, equipped with a IV Fl epi-fluorescence system. The light source was an Osram HB50 mercury lamp; the filters used were: excitor-interference BP405/8; barrier-LP418 and dichromatic beam splitter-FT420. Photomicrographs were taken on Ektachrome ASA 160 tungsten film with a MC63 photomicrographic camera.

Results

Action potential conduction

Following supramaximal stimulation of the hypogastric nerve trunk, several action potentials (APs) were recorded from a small bundle of vas deferens nerves. The latency (up to 40 ms) and amplitude (up to 0.5 mV) of the extracellularly-recorded APs varied, reflecting the different diameters and conduction velocities of the fibres present. The pattern of AP discharge could be changed by altering the stimulation voltage and/or pulse width so that it was often possible to record a single all-or-none biphasic AP of approximately 2 ms duration, representing conduction in either one, or possibly a few, nerve fibres with the same latency and voltage threshold.

Perfusion with verapamil ($0.5-2 \times 10^{-4}$ M), diltiazem ($1-8 \times 10^{-4}$ M), amlodipine ($0.5-2 \times 10^{-4}$ M), cobalt ($2-6 \times 10^{-2}$ M), the local anaesthetic, lignocaine ($0.1-2 \times 10^{-3}$ M) but not with nifedipine ($1-5 \times 10^{-3}$ M), inhibited the amplitude and number of APs in a concentration-dependent manner (as shown for verapamil and nifedipine in Figure 1). The Ca^{2+} channel antagonists initially increased the latency, then reduced the amplitude and finally abolished the AP. The order of potency for the organic Ca^{2+} channel antagonists was amlodipine > verapamil > diltiazem. Lignocaine was less potent than any of these but was more effective than cobalt.

Transmitter release

(1) *Excitatory junction potentials* The smooth muscle of the vas had a resting membrane potential of -55 ± 0.7 mV ($n = 62$) and frequently showed spontaneous e.j.ps (5 ± 0.4 mV in amplitude, $n = 46$ from 6 different preparations). Field stimulation (trains of 10 pulses at 1 and 2 Hz, 0.1–0.5 ms pulse width, supramaximal voltage) evoked e.j.ps 5 ± 0.6 mV in amplitude ($n = 20$ cells) which facilitated at 1 Hz (up to 25 mV) and summated at 2 Hz. Where summation of e.j.ps reached threshold a muscle AP and contraction were produced. These characteristics are similar to those previously reported for e.j.ps in this tissue (Burnstock & Holman, 1961). Verapamil ($1-2 \times 10^{-4}$ M), diltiazem ($1-5 \times 10^{-4}$ M), amlodipine ($0.5-1 \times 10^{-4}$ M), cobalt ($1-10^{-3}$ M) but not nifedipine (5×10^{-4} M) each inhibited evoked e.j.ps (as shown for verapamil and cobalt in Figures 2 and 3), reducing and then abolishing their amplitude in a concentration-dependent manner. The order of potency was amlodipine > verapamil > diltiazem. Cobalt inhibited e.j.ps at a lower concentration than that ($2-6 \times 10^{-2}$ M) required to block AP conduction in the hypogastric nerves. Noticeably, verapamil, diltiazem and amlodipine were more potent in preventing the muscle contractions than in inhibiting the e.j.ps.

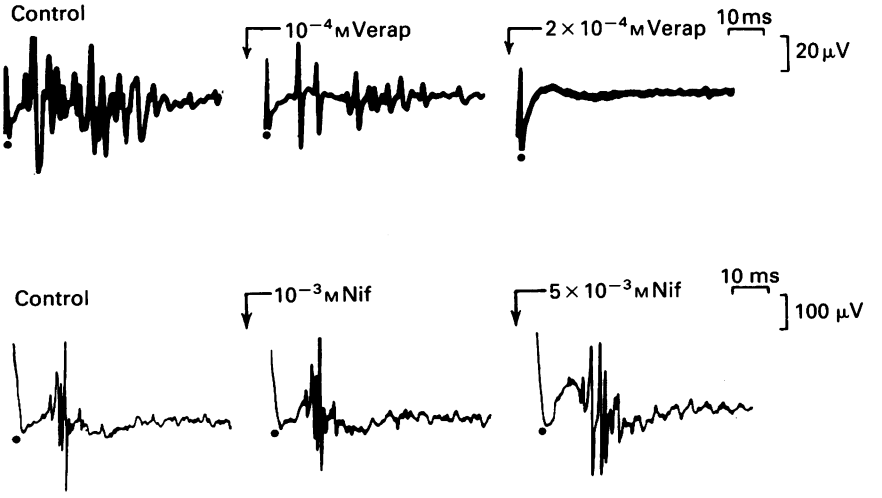

Figure 1 The effects of verapamil (Verap $1-2 \times 10^{-4}$ M) and nifedipine (Nif, $1-5 \times 10^{-3}$ M) on action potential (AP) conduction evoked by stimulation (supramaximal voltage, 0.5 Hz, 0.2 ms) of the guinea-pig vas deferens nerve. Verapamil, but not nifedipine, inhibited the amplitude and number of APs. The stimulus artefact is indicated in each panel by a dot (•).

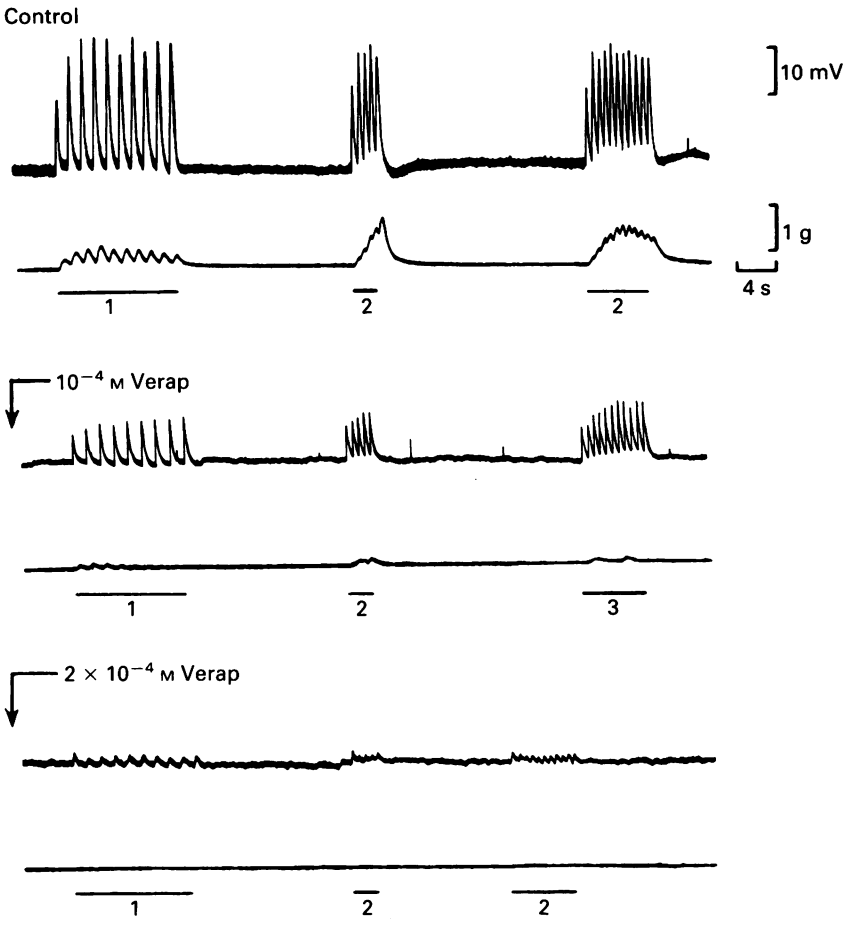

Figure 2 The concentration-dependent inhibitory action of verapamil (Verap, $1-2 \times 10^{-4}$ M) on excitatory junction potentials (e.j.ps; upper traces) and accompanying contractions evoked by field stimulation (supramaximal voltage, 1 and 2 Hz, 0.2 ms) of the guinea-pig vas deferens. The duration of stimulation is indicated by the horizontal bars and the frequency (Hz) by the numbers underneath.

(2) *^3H overflow following preincubation with [^3H]-noradrenaline* In control (i.e. unstimulated) tissues the ^3H overflow into the superfusate declined exponentially with time, a steady level occurring after approximately 2 h. Electrical field stimulation (60 pulses at 1, 5, 10 and 20 Hz, 0.5 ms pulse width, supramaximal voltage) in the absence of other drugs produced a TTX (1×10^{-6} M)-sensitive significant increase in the overflow of ^3H.

Separation and measurement of the tritiated compounds in the superfusate by chromatography revealed that the major constituents in the resting ^3H overflow were VMA and MOPEG, DOPEG and NA, and that stimulation (300 pulses at 20 Hz, 0.5 ms pulse width, supramaximal voltage) released mainly NA (Figure 4a and b). Cobalt (2×10^{-3} M), while having little effect on the resting ^3H output, significantly inhibited stimulation-evoked overflow of ^3H at each frequency (Figure 5). Verapamil ($0.1-1 \times 10^{-4}$ M), diltiazem ($0.5-1 \times 10^{-4}$ M), amlodipine ($0.05-1 \times 10^{-4}$ M) and nifedipine ($1-5 \times 10^{-4}$ M) increased, significantly, the resting overflow of ^3H (as shown for amlodipine in Figure 6). As a result, the amplitudes of the responses to nerve stimulation were insignificant in comparison with the increased resting overflow. Hence, the effects of the organic Ca^{2+} channel antagonists on the evoked overflow could not be determined easily. In contrast to that produced by

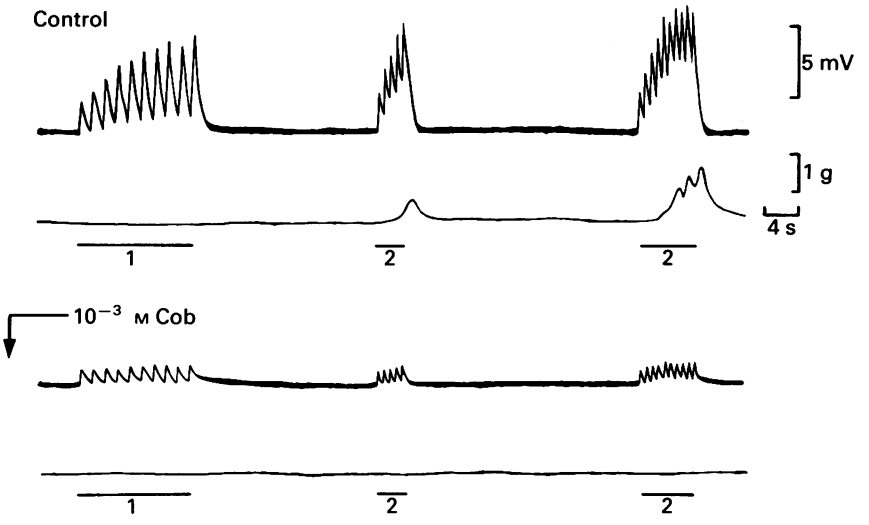

Figure 3 The inhibitory action of cobalt (Cob, 1×10^{-3} M) on excitatory junction potentials (e.j.ps) (upper traces) and accompanying contractile activity evoked by field stimulation (supramaximal voltage, 1 and 2 Hz, 0.5 ms) of the guinea-pig vas deferens. The duration of stimulation is indicated by the horizontal bars and the frequency (Hz) by the numbers underneath.

field stimulation, the principal constituent of the increased resting overflow produced by amlodipine and verapamil, was [^{3}H]-DOPEG (as shown for amlodipine in Figure 7). The amounts of VMA and MOPEG, DOMA, NMN or NA, itself, were not affected significantly by verapamil or amlodipine.

The source of the increased resting ^{3}H overflow was examined in animals pretreated with 6-OHDA to destroy the sympathetic nerve terminals; the effectiveness of this treatment was confirmed by the absence of fluorescence. Following 6-OHDA pretreatment, verapamil (1×10^{-4} M) had little effect on the resting overflow of ^{3}H. The increased ^{3}H overflow produced by the Ca^{2+} channel antagonists was independent of extracellular calcium. Removal of this ion from the Krebs solution without further ionic compensation failed to affect the action of verapamil (1×10^{-4} M).

(3) *^{3}H overflow following preincubation with [^{3}H]-adenosine* In contrast to its effects in tissues preincubated with [^{3}H]-NA, verapamil (1×10^{-4} M) had little effect on the resting overflow of ^{3}H when [^{3}H]-adenosine was used. The amount of ^{3}H released by field stimulation, following preincubation with [^{3}H]-adenosine, was poor, compared with that following pretreatment with [^{3}H]-NA. Accordingly, the conditions of field stimulation adopted (400–2000 pulses at 20 Hz, supramaximal voltage, 0.5 ms pulse width) were more severe. Moreover, since the evoked overflow of ^{3}H from this tissue following field stimulation arises largely from the muscle itself (Westfall *et al.*, 1978), a hypertonic Krebs containing sucrose (12.5%), α,β-meATP and prazosin (both 5×10^{-6} M) was used to prevent muscle contractions induced by ATP and NA respectively, released from the sympathetic nerves (Sneddon & Westfall, 1984). The ^{3}H overflow in response to successive periods of field stimulation declined. Accordingly, separate tissues from the same animal were used for control and drug treatments. The amounts of ^{3}H in the resting overflow from each tissue varied (as in Figures 8 and 9). These variations, which were also observed between control tissues independently of the presence of Ca^{2+} channel antagonists or TTX, remain unexplained.

Field stimulation produced a TTX (2×10^{-6} M)-sensitive increase in ^{3}H overflow (Figure 8). Diltiazem (5×10^{-4} M) and cobalt (2×10^{-3} M, Figure 9) inhibited field stimulation-evoked overflow of ^{3}H. Nifedipine (5×10^{-4} M) was again ineffective.

Discussion

The results clearly indicate that Ca^{2+} channel antagonists have important prejunctional effects. The high concentrations of verapamil, diltiazem and amlodipine required to block AP conduction in the vas deferens nerves suggest a non-specific, possibly a local

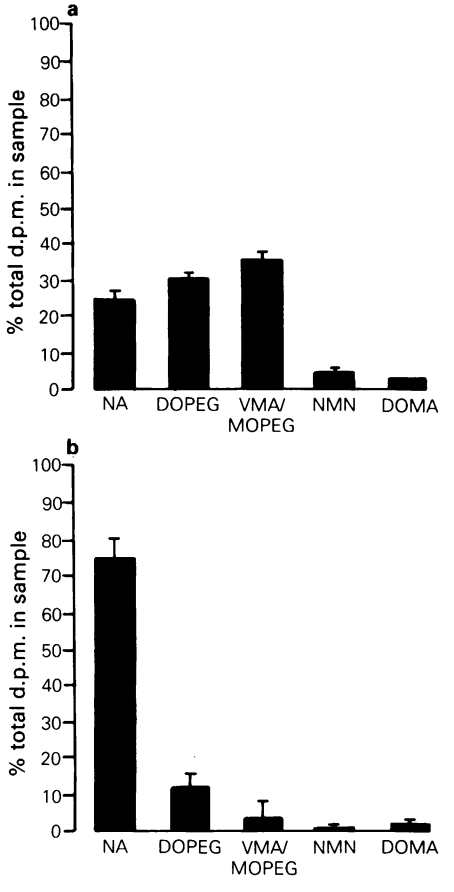

Figure 4 The relative amounts (vertical lines show s.e.mean, $n = 12$) of [^{3}H]-noradrenaline ([^{3}H]-NA) and its metabolites, expressed as a percentage of the total disintegrations per minute per sample (d.p.m. per sample) in the ^{3}H overflow (a) at rest and (b) following field stimulation (300 pulses at 20 Hz, supramaximal voltage, 0.5 ms) of the guinea-pig vas deferens. At rest ^{3}H-labelled 4-hydroxy,3-methoxymandelic acid (VMA) and 4-hydroxy,3-methoxyphenylglycol (MOPEG), 3,4-dihydroxyphenylglycol (DOPEG) and NA were the major constituents; following field stimulation [^{3}H]-NA itself was the principal product. NMN = normetanephrine and DOMA = 3,4-dihydroxymandelic acid.

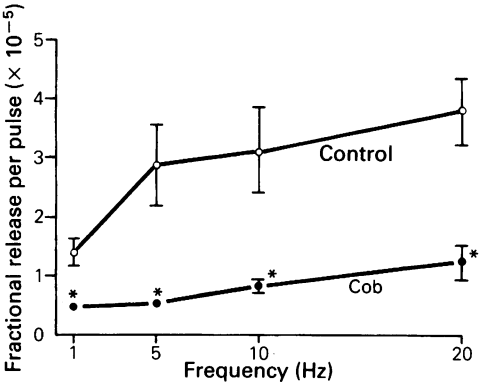

Figure 5 The inhibitory action of cobalt (Cob, 2×10^{-3} M, $n = 3$) expressed as the fractional release per pulse of stimulation, on ^{3}H overflow evoked by field stimulation (60 pulses at 1, 5, 10 and 20 Hz, supramaximal voltage, 0.5 ms) of the guinea-pig vas deferens preincubated with [^{3}H]-noradrenaline ([^{3}H]-NA). Cobalt significantly inhibited ^{3}H overflow at each frequency. Vertical lines show s.e.mean.

anaesthetic, effect mediated via an inhibition of Na^+ conductance rather than on Ca^{2+} channels, themselves. Indeed verapamil, diltiazem and amlodipine were each more potent than lignocaine, whereas nifedipine, devoid of local anaesthetic action, had no effect on AP conduction. The TTX-sensitive nature of the AP in the nerves concerned is in keeping with this proposal and a local anaesthetic effect has already been claimed for verapamil in somatic nerves (Hay & Wadsworth, 1982).

Since both NA and ATP are released simultaneously by nerve stimulation, probably from the same vesicle in the guinea-pig vas deferens (Sneddon & Westfall, 1984), both transmitter pools were labelled separately to allow the effects of Ca^{2+} channel antagonists to be measured.

Verapamil, diltiazem and amlodipine inhibited release, as measured by their effect on evoked e.j.ps, only in doses similar to those which blocked AP conduction in nerves. Hence, it is likely that transmitter release was inhibited because of the failure of the AP to reach the nerve terminal rather than by preventing Ca^{2+} entry at the terminal itself. Nifedipine failed to block neuronal conduction and was also ineffective on transmitter release. Unlike the organic Ca^{2+} channel antagonists that affected preterminal conduction, cobalt blocked the release of both transmitter substances as indicated by a reduction in e.j.p. amplitude and ^{3}H overflow, at a concentration which failed to affect AP conduction. In this case, the site of action of cobalt appears to be at or near the nerve terminal, rather than at the preterminal nerves from where APs were recorded. Two possible mechanisms for the inhibition of release could be involved. First, cobalt, which in this concentration ($1-2 \times 10^{-3}$ M) may be selective for Ca^{2+} channels (Edwards, 1982), could have blocked Ca^{2+} entry if this ion is involved in AP conduction (Cunnane & Stjärne, 1984) at the nerve terminal. Secondly, cobalt could also have prevented Ca^{2+}

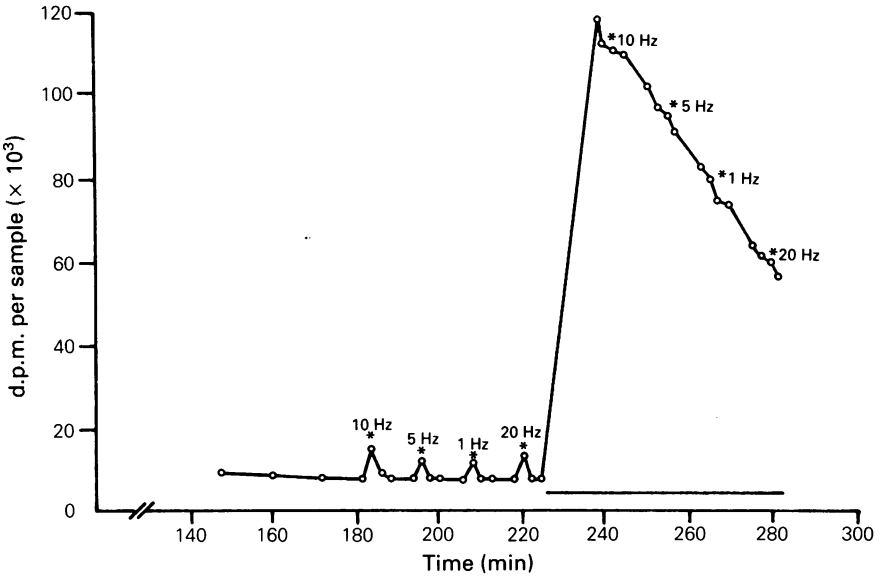

Figure 6 The typical effects of amlodipine (5×10^{-5} M for the period indicated by the horizontal bar), expressed as d.p.m. per sample, on ^3H overflow evoked by field stimulation (60 pulses at 1, 5, 10 and 20 Hz, supramaximal voltage, 0.5 ms) of the guinea-pig vas deferens, preincubated with [^3H]-noradrenaline ([^3H]-NA). Amlodipine increased the resting ^3H overflow sufficiently to mask the effect of field stimulation at each frequency.

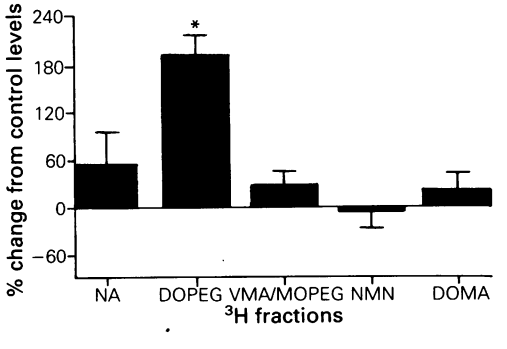

Figure 7 The effect of amlodipine (5×10^{-6} M, $n = 3$) expressed as the percentage change from control levels, on the resting overflow of [^3H]-noradrenaline ([^3H]-NA) and its metabolites from the guinea-pig vas deferens. Amlodipine selectively increased the levels of [^3H]-DOPEG. Vertical lines show s.e.mean. For abbreviations see legend to Figure 4.

entry at the transmitter release site itself.

Although the ability to detect ^3H in response to field stimulation following pre-incubation with [^3H]-adenosine is compatible with a transmitter role for the nucleotide, the amount of overflow was small. Those Ca^{2+} channel antagonists which modified the release of one transmitter did so for the other also. On the other hand, nifedipine was ineffective in preventing the stimulation-evoked overflow of ^3H following incubation with either [^3H]-NA or [^3H]-adenosine. This result suggests that the release process for each transmitter is similar; that both require Ca^{2+} and are similarly affected by Ca^{2+} channel antagonists. If both the non-adrenergic transmitter and NA are released from the same nerve (Burnstock & Sneddon, 1984; Sneddon & Westfall, 1984) the action potential should have equal access to each transmitter. The low amount of ^3H released by field stimulation may simply reflect the relatively small amount of nucleotide present in the nerves if, as suggested (Stjärne & Åstrand, 1984), the NA:ATP ratio is approximately 50:1. On the other hand, the labelling procedure may be comparatively ineffective for the nucleotide; if so, the mechanism of its storage and release may differ from that of NA. This merits further examination.

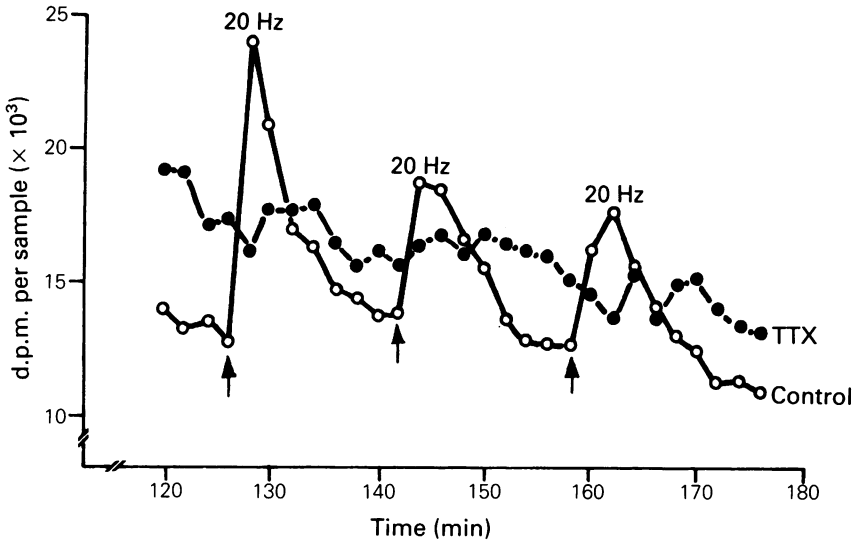

Figure 8 The inhibitory action of tetrodotoxin (TTX, 2×10^{-6} M) in a typical experiment on ^3H overflow, expressed as d.p.m. per sample, evoked by field stimulation (2000 pulses at 20 Hz, supramaximal voltage, 0.5 ms) of the guinea-pig vas deferens preincubated with [^3H]-adenosine and in the presence of sucrose (12.5%), α,β-meATP and prazosin (both 5×10^{-6} M) to inhibit contractions. Tissues were stimulated simultaneously; commencement of each period of stimulation is indicated by an arrow (↑).

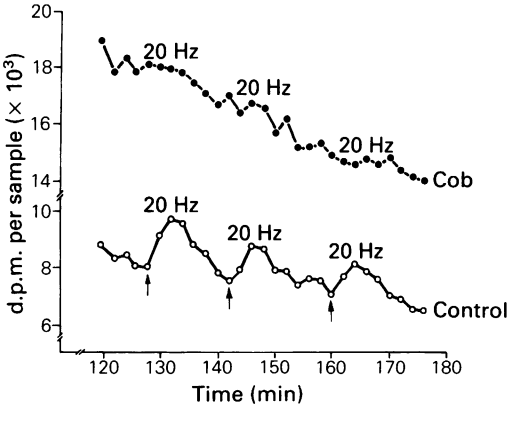

Figure 9 The inhibitory action of cobalt (Cob, 2×10^{-3} M compared with control), in a typical experiment on ^3H overflow, expressed as d.p.m. per sample, evoked by field stimulation (2000 pulses at 20 Hz, supramaximal voltage, 0.5 ms) of the guinea-pig vas deferens preincubated with [^3H]-adenosine and in the presence of sucrose (12.5%), α,β-meATP and prazosin (both 5×10^{-6} M) to inhibit contractions. Tissues were stimulated simultaneously; commencement of each stimulation period is indicated by an arrow (↑).

The enhanced release of ^3H by the Ca^{2+} channel antagonists from sympathetic nerve terminals, following preincubation with [^3H]-NA has been previously reported in other tissues (e.g. canine saphenous vein (Takata & Kato, 1984), rat tail artery and guinea-pig vas deferens (Wolchinsky & Zsotér, 1985)). This could have arisen either from a reserpine-like or a tyramine-like action. The fast onset of release of [^3H]-DOPEG independently of external Ca^{2+} is reminiscent of a tyramine-like effect. Moreover the effect was not seen when [^3H]-adenosine was used instead of [^3H]-NA. On the other hand, the granular uptake mechanism for adenosine is reserpine-insensitive and may be independent of that for NA (Winkler *et al.*, 1981). Although its basis was not investigated, this mechanism could account for the increased response to evoked transmitter observed in the presence of low concentrations of verapamil (French & Scott, 1981; Bornstein *et al.*, 1985) and nifedipine (Bornstein *et al.*, 1985).

From a study of the effectiveness of Ca^{2+} channel antagonists pre- and postjunctionally it is obvious that the Ca^{2+} channels at these sites differ. The organic Ca^{2+} channel antagonists are potent postjunctionally, requiring high concentrations before conduction of the nerve AP and release of transmitter are inhibited. Although it is unlikely that this prejunctional activity

is important with the low doses used therapeutically, this apparent selectivity for the different Ca^{2+} channels opens the possibility of developing Ca^{2+} channel antagonists which act preferentially on nerves.

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