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Relaxations of the isolated portal vein of the rabbit induced by nicotine and electrical stimulation

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Summary

1. A pharmacological analysis of the inhibitory innervation of the isolated portal vein of the rabbit has been made.

2. In untreated preparations, transmural stimulation elicited a long-lasting relaxation at low frequencies (0·2–1 Hz); at higher frequencies a contraction followed by a prolonged after-relaxation occurred. Tetrodotoxin abolished the contractions but a higher dose was required to abolish the relaxations. Veratrine lowered the threshold of stimulation for producing relaxations in the untreated vein. The relaxations were unaffected by hyoscine or hexamethonium. They were reduced or altered by antagonists of α-adrenoceptors for catecholamines and by adrenergic neurone blockade. They were sometimes slightly reduced by antagonists of β-adrenoceptors.

3. In the presence of antagonists of α-adrenoceptors, electrical stimulation elicited relaxations which increased with frequency of stimulation and became maximal at 20–30 Hz. These relaxations were partially reduced by antagonists of β-adrenoceptors, or by adrenergic neurone block; the antagonisms were more pronounced at the higher frequencies of stimulation. Noradrenaline also caused relaxations which were abolished by β-adrenoceptor blocking drugs. Cocaine increased the sensitivity to noradrenaline by 7–8 fold after α-adrenoceptor blockade but had little or no effect on the relaxations induced by electrical stimulation at high frequencies.

4. In the presence of antagonists of α- and β-adrenoceptors, or adrenergic neurone blocking agents, or in veins taken from rabbits pretreated with reserpine, electrical stimulation elicited rapid relaxations which were greatest at 20–30 Hz. These relaxations were increased by veratrine and abolished by tetrodotoxin or by storing the vein for 9 days at 4° C. They were unaffected by antagonists of acetylcholine, or by dipyridamole.

5. Prostaglandins E_1, E_2 and $F_{2\alpha}$ inhibited contractions elicited by electrical stimulation and noradrenaline, but in higher doses caused contractions themselves.

6. Nicotine (10^{-6}–10^{-5} g/ml) relaxed the portal vein; higher concentrations elicited mixed inhibitory and excitatory effects. All these effects were abolished

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by tetrodotoxin, cocaine, hexamethonium or storage. The contractor effects were abolished by drugs or procedures that blocked adrenergic mechanisms.

7. The relaxations produced by nicotine in untreated preparations and in veins from rabbits pretreated with reserpine were mediated mainly by a non-adrenergic non-cholinergic nervous mechanism. Relaxations induced by nicotine in the presence of antagonists of α-adrenoceptors were only partially antagonized by antagonists of β-adrenoceptors.

8. It was concluded that all the effects of nicotine and transmural stimulation were mediated by nerves. Part of the inhibitory effects was mediated by non-adrenergic, non-cholinergic nerves.

Introduction

Transmural electrical stimulation of the isolated portal vein of the rabbit caused contractions of the longitudinal muscle by excitation of post-ganglionic sympathetic nerves (Hughes & Vane, 1967). Abolition of the contraction by various blocking agents revealed a relaxation of the vein which was mediated partially by sympathetic nerves and partially by a non-adrenergic, non-cholinergic nervous mechanism. We have now examined this relaxation further and we will present evidence that nicotine also stimulates the non-adrenergic inhibitory nerves in this preparation.

Methods

Male albino rabbits weighing 2–3 kg were killed by breaking the neck. The portal vein was removed and suspended in an isolated organ bath. Isometric tension of the longitudinal muscle was recorded, as described by Hughes & Vane (1967) with an isometric transducer (tensile sensor, Type S.T.I. Ether-Langham Thompson Ltd.) and a potentiometric millivolt recorder (Texas Instruments Servo Riter). The vein was bathed in 10–50 ml of Krebs solution at 37° C, gassed with 95% oxygen and 5% carbon dioxide; the Krebs solution had the following composition (mM): NaCl 118, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·17, KCl 4·7, $NaHCO_3$ 25, glucose 5·6.

For transmural stimulation, platinum electrodes were positioned on opposite sides of the tissue (Hughes & Vane, 1967). The vein was stimulated with rectilinear pulses of 1 ms duration and supramaximal voltage (15–18 V across the tissue). The stimulation was presented at different frequencies, either for a fixed period of time, or for a set number of pulses.

To deplete the catecholamine stores in the portal vein, reserpine (1 mg/kg) was injected intramuscularly into the rabbit on each of 2 days. The rabbits were kept in a warm, dark environment and disturbed as little as possible until they were killed on the third day.

The following agonists were used: acetylcholine perchlorate (British Drug Houses), adenosine and its mono-, di-, and triphosphates (B.D.H.), dopamine hydrochloride (Sigma), (±)-isopropylnoradrenaline sulphate (B.D.H.), (−)-nicotine hydrogen tartrate (B.D.H.), (−)-noradrenaline bitartrate (B.D.H.), prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn), veratrine alkaloids (B.D.H.).

The following antagonists were used: bethanidine sulphate (Burroughs Wellcome), bretylium tosylate (B.W.), cocaine hydrochloride (B.D.H.), dipyridamole (Persantin, Boehringer Ingelheim), ergotamine tartrate (Sandoz), guanethidine

sulphate (Ciba), hexamethonium bromide (May and Baker), hyoscine hydrobromide (B.D.H.), morphine sulphate (B.D.H.), 1-(*p*-nitrophenyl-2-isopropylamine)ethanol hydrochloride (±INPEA), physostigmine sulphate (eserine, B.D.H.), phentolamine methanesulphate (Ciba), reserpine (Ciba), tetrodotoxin (Sankyo-Tokyo).

All drugs were freshly diluted in saline (0·9% w/v) from stock solutions. The doses of the salts are expressed in terms of base and as final bath concentration (g/ml), unless otherwise stated. Agonists were added directly to the bathing fluid in volumes of less than 0·4 ml; they were left in the bath for 1 min and then washed out by overflow.

Results

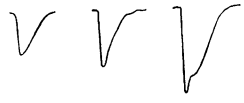
Characteristics of the relaxation induced by transmural stimulation

Table 1 illustrates the shapes and time courses of the relaxations which can be produced in the portal vein by transmural stimulation. Type 1 relaxation (untreated preparations) was most easily demonstrated in preparations in which the basal tension increased spontaneously during the initial equilibration period (Hughes & Vane, 1967). The most striking feature of these relaxations and after-relaxations was the time-course for recovery; after a stimulation lasting for 30 s it sometimes took up to 20 min for the vein to regain its resting tension.

Type 2 relaxation (after α-adrenoceptor block) had a shorter time course (6–12 min). A biphasic response could often be elicited under conditions of partial α-adrenoceptor block (Fig. 1); this consisted of a quick relaxation followed by a slower and more prolonged relaxation. Propranolol ($2–5 \times 10^{-6}$ g/ml) abolished the second prolonged relaxation, leaving the quick relaxation only; this was similar to the inhibitions obtained in preparations treated with adrenergic neurone blocking agents, or in preparations from rabbits pretreated with reserpine (type 3 relaxation).

The rapid type 3 relaxation which was not mediated by adrenergic nerves is shown in Fig. 2. It occurred at all frequences of stimulation from 0·2 Hz and

TABLE 1. *Different types of inhibitory response elicited by transmural stimulation*

	0·5 Hz	2–4 Hz	10–30 Hz
Type 1 Untreated preparations 10–20 min time course.			
Type 2 Vein treated with antagonists of α-adrenoceptors. 6–12 min time course.			
Type 3 Veins treated with either (*a*) antagonists of α- and β-adrenoceptors (*b*) adrenergic neurone blocking agents, or (*c*) veins from rabbits pretreated wih reserpine 1·5–4 min time course.			

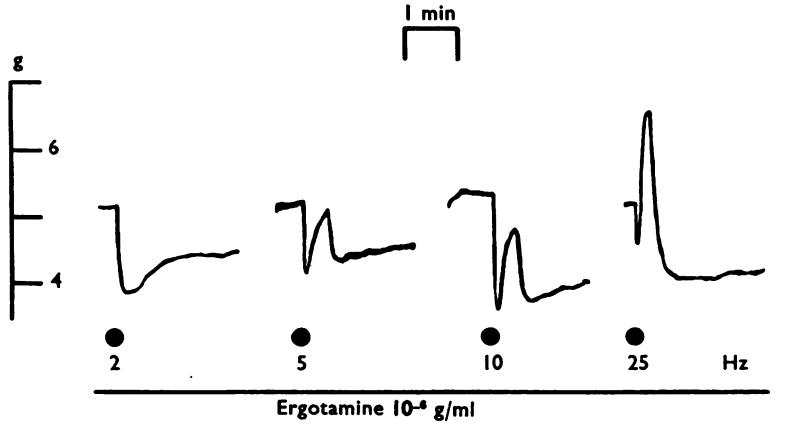


FIG. 1. Responses of portal vein to electrical stimulation (at dots, 2, 5, 10 and 25 Hz for 20 s) at fast paper speed, during incomplete α-adrenoceptor block with ergotamine (1×10^{-6} g/ml). Note the initial quick relaxation at frequencies of 5, 10 and 25 Hz, and the second, longer-lasting relaxation which persisted after cessation of the stimulus. Time scale 1 min; vertical scale in g.

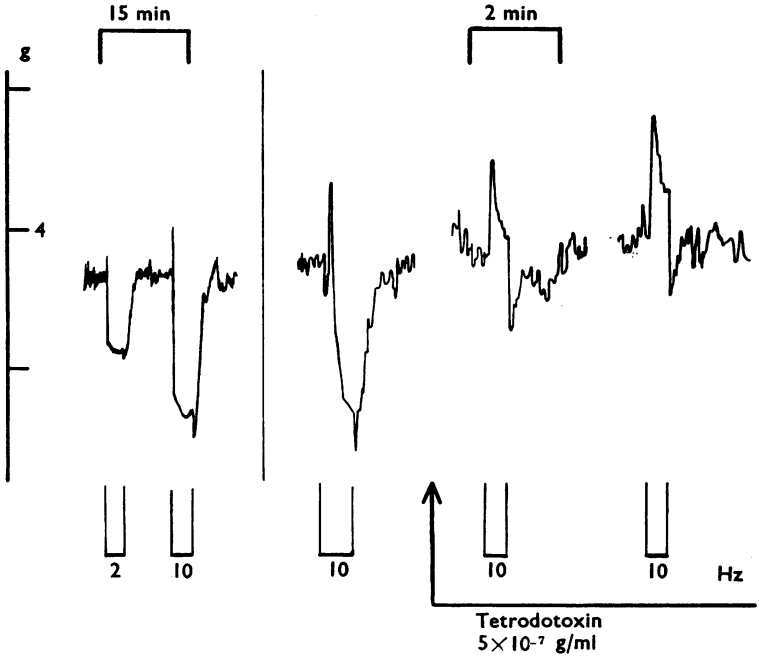


FIG. 2. Portal vein treated with bretylium (5×10^{-6} g/ml). First panel: effect of stimulation at 2 and 10 Hz for 3 min (at ⊔); the relaxation is maintained throughout the stimulation. Time scale 15 min. Second panel: relaxations induced by electrical stimulation (10 Hz for 30 s at ⊔) at fast paper speed. Tetrodotoxin (5×10^{-7} g/ml) abolished the relaxation which occurred during stimulation but did not reduce the initial contraction or the after-stimulus relaxation. Time scales 15 min and 2 min; vertical scale in g.

became maximal at 20–30 Hz. The peak effect obtainable at any one frequency occurred within 80–120 pulses, but the relaxation was maintained for as long as the stimulus was continued.

Effects of nicotine

The actions of nicotine depended on the concentration applied and on the basal tension of the vein. In preparations with a high resting tension, low concentrations of nicotine (10^{-6}–10^{-5} g/ml; twenty-three experiments) always caused a relaxation of the vein; this effect rapidly waned when the nicotine was washed out of the bath. At higher concentrations (10^{-5}–10^{-4} g/ml) nicotine either contracted the vein or elicited a biphasic response.

In preparations with a low basal tone, the contractor effects of nicotine predominated at all concentrations. However, the contraction was not maintained; the tension reached a maximum within 30–40 s and thereafter declined, returning to baseline within 6–10 min. The onset of the response was extremely rapid, the latent period rarely exceeding two seconds. When repeated doses of nicotine were given at intervals of less than 15–20 min apart, the tissue became insensitive to the drug, but remained reactive to electrical stimulation.

Effects of blocking or potentiating nervous mechanisms

Both the excitatory and inhibitory effects of nicotine were reduced by non-anaesthetic concentrations of cocaine (10^{-7}–10^{-6} g/ml) which potentiated contractions induced by transmural stimulation or noradrenaline. Relaxations elicited by stimulation at low frequency and the after-relaxations associated with stimulation at high frequencies (type 1 relaxation) were unaffected by these concentrations of cocaine. Anaesthetic concentrations of cocaine (10^{-5} g/ml) reduced the basal tension and abolished all the effects of transmural stimulation and nicotine whilst contractions induced by noradrenaline remained potentiated.

Tetrodotoxin (10^{-7}–5×10^{-7} g/ml) abolished contractions induced by nicotine or transmural stimulation without reducing the effects of noradrenaline. Relaxations induced by nicotine were also abolished without any reduction in relaxations induced by isoprenaline. However, the type 1 relaxations were only reduced by 30–50% at these concentrations of tetrodotoxin (four experiments); higher concentrations of tetrodotoxin (5×10^{-6} g/ml; three experiments) abolished all the responses to transmural stimulation without changing the effects of noradrenaline or isoprenaline (Fig. 3).

When the portal vein was stored in Krebs solution at 4° C for 9 days, nicotine and transmural stimulation no longer produced a response (five experiments). However, these preparations were more sensitive to noradrenaline than before storage and the concentration of noradrenaline required to elicit a 50% maximum response was halved. Isoprenaline still relaxed the preparation which had been stored, but there was no increase in sensitivity.

Veratrine enhances the effects of transmural electrical excitation of the portal vein without changing the effects of noradrenaline (Hughes & Vane, 1967). The relaxations and contractions induced by nicotine were also enhanced by veratrine (10^{-6} g/ml; three experiments).

Effects of antagonists of β-adrenoceptors for catecholamines

Propranolol (5×10^{-7}–1×10^{-5} g/ml; six experiments) and INPEA had no effect on the after-relaxation following electrical stimulation, although the relaxation induced by low frequency stimulation was slightly reduced after prolonged contact with higher concentrations of these antagonists. However, these antagonisms were small and could not be demonstrated at concentrations (propranolol, 10^{-6} g/ml; INPEA, 5×10^{-6} g/ml) which abolished the effects of isoprenaline.

Relaxations induced by nicotine were slightly reduced by INPEA (10^{-5} g/ml; five experiments, Fig. 4) but were greatly reduced or abolished by propranolol (2×10^{-6} g/ml; three experiments). The block induced by propranolol of the relaxation elicited by nicotine was reversed 60–90 min after washing out the antagonist, whereas relaxations induced by isoprenaline were not restored within this time.

Effects of antagonists of α-adrenoceptors for catecholamines

(*a*) *In the untreated vein.* Phentolamine (5×10^{-6} g/ml) or ergotamine (5×10^{-6} g/ml) abolished the excitatory effects of nicotine, noradrenaline and transmural stimulation, after which all these stimuli elicited relaxations which were dependent on concentration of drug or frequency of stimulation.

(*b*) *Effects of other substances in the presence of α-adrenoceptor block.* In preparations which had been exposed to phentolamine (5×10^{-6} g/ml) or ergotamine (5×10^{-6} g/ml) for 1–2 h, low concentrations of cocaine (10^{-6} g/ml; four experiments) substantially increased the size and duration of the relaxations induced by

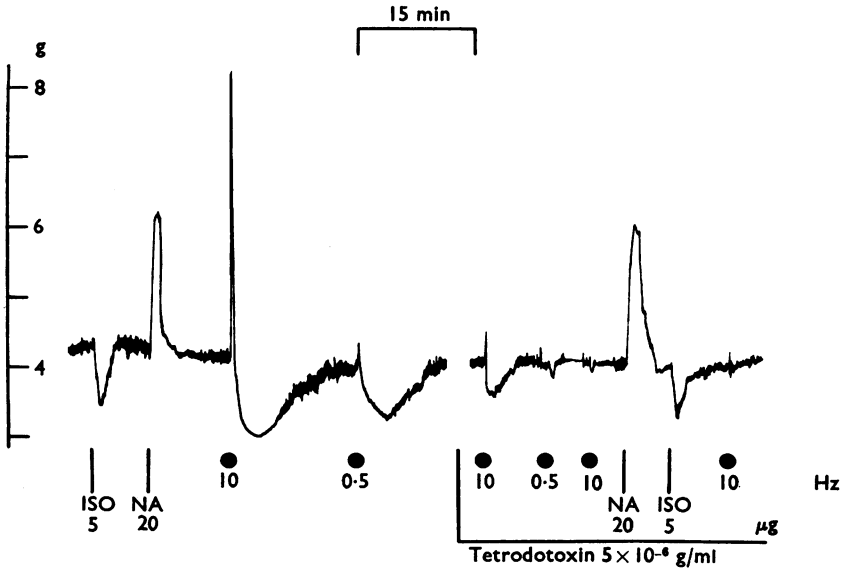


FIG. 3. Complete block of the effects of electrical stimulation by tetrodotoxin. Untreated vein. Electrical stimulation at 0·5 Hz (at dots, 100 pulses) elicited a prolonged relaxation; a contraction followed by an after-relaxation occurred when the frequency was increased to 10 Hz (at dots, 100 pulses). Noradrenaline (NA, 20 μg/40 ml bath) had purely excitatory effects, and isoprenaline (ISO, 5 μg/40 ml bath) purely inhibitory effects. Tetrodotoxin (5×10^{-6} g/ml) abolished all the effects of electrical stimulation but the responses to noradrenaline and isoprenaline were little affected. Time scale 15 min; vertical scale in g.

noradrenaline, but reduced by 40–60% those elicited by nicotine (10^{-6}–10^{-4} g/ml) (Fig. 5). Relaxations induced by electrical stimulation at frequencies above 8 Hz were slightly increased in duration (30%) in two experiments but were unaffected in two others. Relaxations elicited by isoprenaline were unchanged.

Tetrodotoxin (5×10^{-7} g/ml; four experiments) abolished the relaxations elicited by nicotine and transmural stimulation without reducing the effects of noradrenaline.

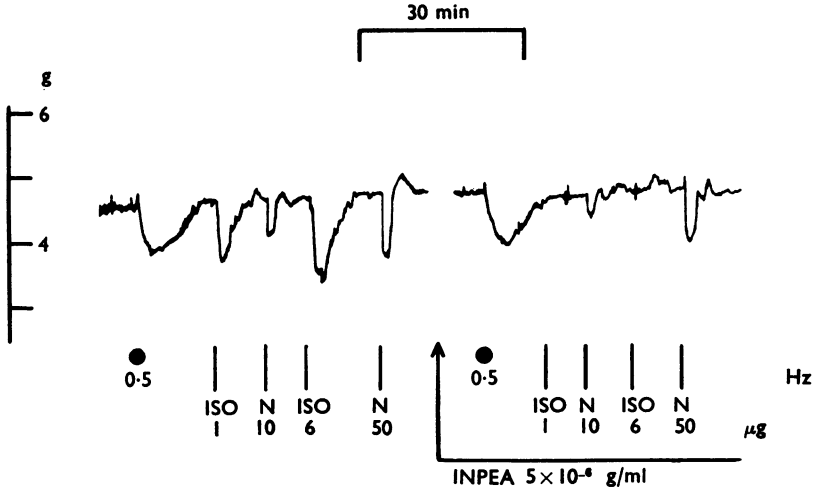


FIG. 4. Effect of INPEA on relaxations of untreated portal vein, induced by electrical stimulation (at dots, 0·5 Hz, 120 pulses), nicotine (N, 10 and 50 μg/10 ml) and isoprenaline (ISO, 1 and 6 μg/10 ml). Ninety minutes after INPEA (5×10^{-6} g/ml) the relaxations elicited by isoprenaline were abolished; however, nicotine-induced relaxations were only reduced by 20–30% and the relaxation caused by stimulation at 0·5 Hz was unaffected. Time scale 30 min; vertical scale in g.

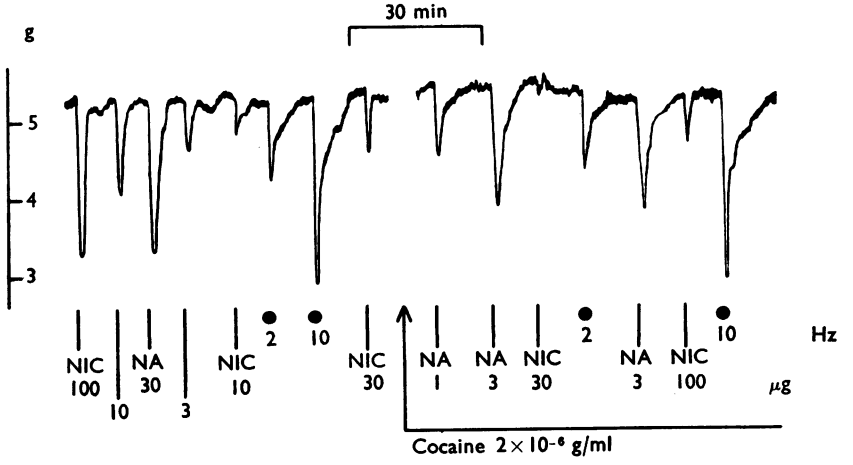


FIG. 5. Portal vein treated with ergotamine (5×10^{-6} g/ml) for 2 h previously. Effect of a non-anaesthetic concentration of cocaine on relaxations produced by electrical stimulation (at dots, 2 and 10 Hz for 20 s), nicotine (NIC, 10, 30 and 100 μg/10 ml) and noradrenaline (NA, 3, 10 and 30 μg/10 ml). After 40 min contact with cocaine (2×10^{-6} g/ml) there was a 7–8 fold increase in sensitivity to noradrenaline but relaxations induced by electrical stimulation were only slightly increased in duration at 10 Hz. Relaxations induced by nicotine were almost completely abolished. Time scale 30 min; vertical scale in g.

Relaxations induced by noradrenaline and isoprenaline were abolished by INPEA (5×10^{-6}–10^{-5} g/ml; fifteen experiments) but the relaxations elicited by electrical stimulation and nicotine were only partially antagonized. The reduction in the effects of electrical stimulation varied from 10–50% and was usually much more pronounced at the higher frequencies of stimulation; there was also a reduction in the duration of effect (Fig. 6). When the concentration of noradrenaline, or isoprenaline, was increased 50–500-fold in these preparations treated with α- and β-adrenoceptor blocking agents there was no further relaxation, but instead a slow, sustained contraction occurred (Fig. 6).

Effects of adrenergic neurone block

(*a*) *In the untreated vein.* During adrenergic neurone block transmural stimulation always elicited a characteristic quick relaxation, provided that there was sufficient basal tone. Thus the contraction induced by electrical stimulation and

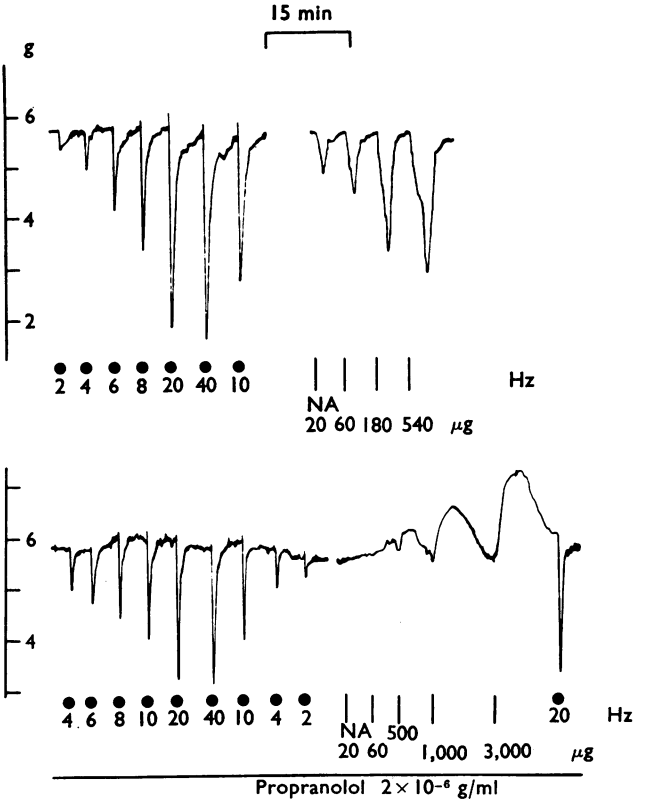


FIG. 6. Effect of propranolol on relaxations elicited by electrical stimulation (at dots, 2–40 Hz for 20 s) and by noradrenaline (NA, 20–3,000 μg/50 ml bath; the recorder chart was stopped during the recovery period in the upper panel). The vein was exposed to ergotamine (5×10^{-6} g/ml) for 2 h before this tracing. Upper panel: relaxations elicited by electrical stimulation (2–40 Hz) and by increasing doses of noradrenaline. Lower panel: 90 min after propranolol (2×10^{-6} g/ml) the relaxations produced by frequencies above 6 Hz were reduced by about 30%, whilst the relaxations induced by the lower frequencies were reduced by less than 10%. Noradrenaline-induced relaxations were completely abolished, and an increase in the dose of noradrenaline now produced a slowly developing contraction which was well maintained. Time scale 15 min; vertical scales in g.

the associated prolonged after-relaxation were abolished by bretylium (5×10^{-6} g/ml; seven experiments), bethanidine ($1–5 \times 10^{-6}$ g/ml; four experiments) or guanethidine ($2–5 \times 10^{-6}$ g/ml; three experiments) (Fig. 7).

All three blocking agents abolished contractions elicited by nicotine (Fig. 7); the effects on the relaxations induced by nicotine were more complex. Bretylium (5×10^{-6} g/ml) almost abolished (80–100%) the nicotine-induced relaxations in both untreated and drug-treated preparations, whereas guanethidine (at concentrations sufficient to abolish all adrenergic responses to nerve stimulation; see Fig. 7) only slightly reduced (10–30%) the relaxations elicited in untreated preparations but caused a more appreciable block (50–60%) of the relaxations in preparations treated with α-adrenoceptor blocking agents. The effects of bethanidine were intermediate between those of bretylium and guanethidine.

The size and duration of the relaxation induced by transmural stimulation was partially reduced by adrenergic neurone blocking agents, but this antagonism was only seen at frequencies of stimulation above 4 Hz.

(*b*) *Effects of other antagonists on veins treated with adrenergic neurone blocking agents.* In four preparations treated with bretylium (5×10^{-6} g/ml) cocaine (10^{-6}

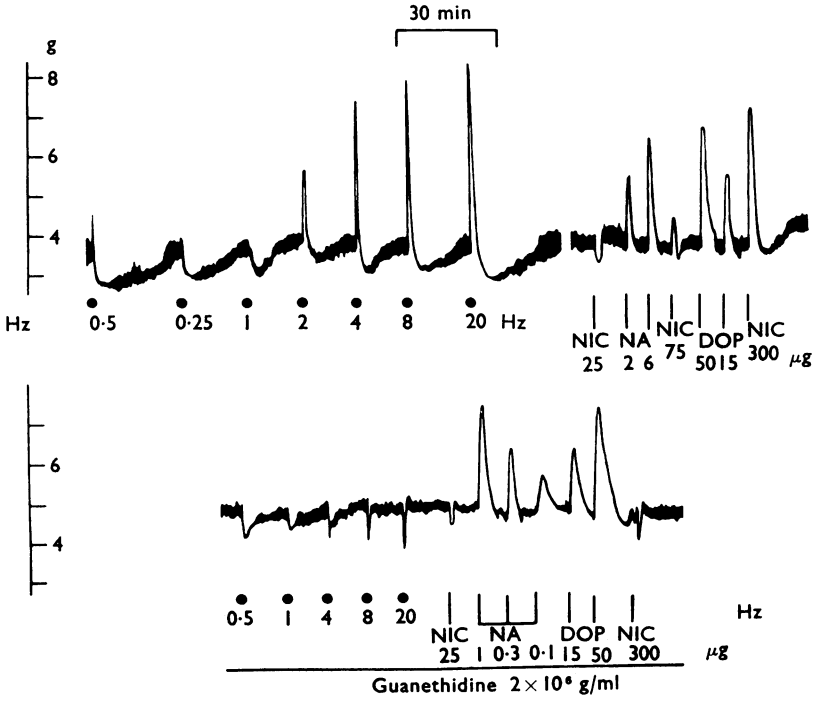


FIG. 7. Effect of guanethidine on responses of portal vein produced by electrical stimulation (at dots, 0·25–20 Hz, 120 pulses), nicotine (NIC, 25, 75 and 300 μg/12 ml), noradrenaline (NA, 2 and 6 μg/12 ml) and dopamine (DOP, 15 and 50 μg/12 ml). Upper panel: electrical stimulation between 0·25 and 1 Hz elicited relaxations at frequencies above 1 Hz; there was a contraction, followed by a relaxation after the stimulus was over. Nicotine caused a relaxation at the low dose (25 μg/12 ml) and a contraction followed by an after-relaxation at the higher doses (75 and 300 μg/12 ml). Lower panel: after 1 h of exposure to guanethidine (2×10^{-6} g/ml) contractions induced by electrical stimulation and nicotine were abolished and only relaxations of short duration could be elicited by these stimuli. There was a 4–6 fold increase in sensitivity to noradrenaline and an increase in the duration of the contractions to dopamine. Time scale 30 min; vertical scales in g.

g/ml) had no effect on the size or duration of the relaxation elicited by transmural stimulation.

Tetrodotoxin (5×10^{-7}–1×10^{-6} g/ml) abolished the relaxation which occurred during electrical stimulation but did not completely prevent the small initial contraction or the after-stimulus relaxation which was seen in four preparations (see Fig. 2); in eight other experiments the initial contraction and after-stimulus relaxation were not seen and tetrodotoxin completely abolished the relaxation induced during stimulation in these preparations.

The relaxation of the portal vein which was induced by nicotine in the presence of adrenergic neurone blocking agents was also abolished by tetrodotoxin (10^{-7} g/ml; two experiments).

Effect of pre-treating rabbits with reserpine

Nicotine in low or high concentrations (10^{-6}–10^{-4} g/ml) had purely inhibitory effects in veins from rabbits pretreated with reserpine (four experiments). These relaxations were reduced by cocaine (10^{-6} g/ml) and abolished by tetrodotoxin (10^{-7} g/ml). The relaxations induced by nicotine in veins from rabbits pretreated with reserpine were antagonized by propranolol, but not by INPEA.

Effects of other drugs

Ganglion blocking agents. Hexamethonium (10^{-6}–10^{-5} g/ml) abolished all responses induced by nicotine, whether in untreated preparations (two experiments), in the presence of ergotamine (5×10^{-6} g/ml; two experiments), INPEA (10^{-5} g/ml; three experiments) or a combination of both (two experiments). Hexamethonium did not affect the relaxations elicited by electrical stimulation.

Hyoscine and anticholinesterases. Acetylcholine usually contracted the portal vein, but occasionally (eight out of forty-six experiments) relaxed it. Hyoscine (10^{-7} g/ml; two experiments) abolished contractions or relaxations elicited by acetylcholine, but did not affect those induced by electrical stimulation, isoprenaline or nicotine (hyoscine 10^{-7}–10^{-6} g/ml; two experiments) (Fig. 8).

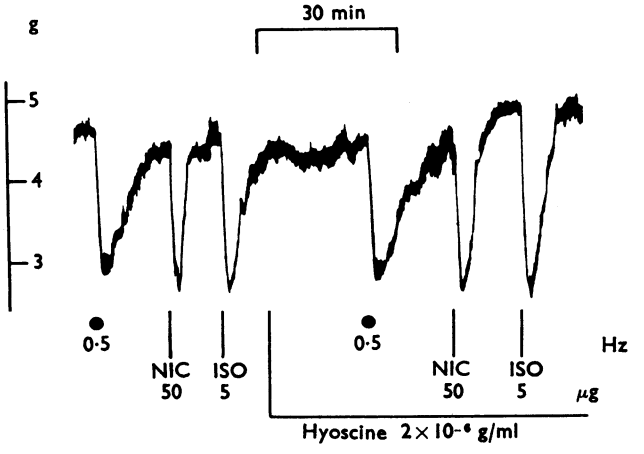


FIG. 8. Failure of hyoscine to affect relaxations of portal vein induced by electrical stimulation (at dots, 0·5 Hz for 100 pulses), nicotine (NIC, 50 μg/40 ml) and isoprenaline (ISO, 5 μg/40 ml). Hyoscine (2×10^{-6} g/ml) did not reduce the response to any of the stimuli. Time scale 30 min; vertical scale in g.

Eserine (10^{-7}–5×10^{-6} g/ml) had no effect on the relaxation following low frequency stimulation (three experiments), on the relaxation elicited by electrical stimulation after α-adrenoceptor blockade (three experiments) or after adrenergic neurone block (three experiments). In two preparations treated with ergotamine (5×10^{-6} g/ml), eserine (10^{-6} g/ml) abolished the relaxations induced by nicotine; this block was reversed when the eserine was removed.

Morphine. This alkaloid did not affect the relaxations induced by low frequency stimulation in untreated preparations (morphine 5×10^{-6} g/ml for 1 h; three experiments) or in those treated with bretylium (three experiments); relaxations elicited by nicotine or isoprenaline were also unaffected.

Dipyridamole (10^{-9}–10^{-6} g/ml) did not alter the relaxations elicited by ATP, adenosine or electrical stimulation in two preparations treated with ergotamine (5×10^{-6} g/ml) and INPEA (10^{-5} g/ml) or in another preparation treated with bretylium (5×10^{-6} g/ml). Concentrations of dipyridamole above 10^{-6} g/ml caused a prolonged relaxation of the isolated vein.

Prostaglandins. Prostaglandin E_1 (10^{-7}–10^{-6} g/ml; six experiments) and prostaglandin E_2 (10^{-8}–10^{-7} g/ml) reduced half-maximal contractions elicited by noradrenaline and electrical stimulation by 30–90%; this inhibition was rapidly reversed by washing the prostaglandins out of the bath. However, prostaglandin E_1, E_2 or $F_{2\alpha}$ did not produce relaxations; indeed, higher concentrations (5×10^{-7}–5×10^{-6} g/ml) induced contractions which increased with the concentration of the drug.

Discussion

We have previously presented strong evidence that transmural electrical stimulation of the isolated portal vein from the rabbit with pulses of up to 1 ms excites nervous tissue *only* (Hughes & Vane, 1967). The present results reinforce this conclusion. Thus the abolition of the effects of transmural stimulation by tetrodotoxin, a substance which specifically abolishes nervous effects in smooth muscle (Gershon, 1967), confirms this view. In addition, storage of the portal vein for 9 days at 4° C abolished the effects of transmural stimulation but not those of noradrenaline or isoprenaline. Presumably during this time the nerve endings degenerated whilst the muscle cells remained viable. This conclusion is supported by the increased sensitivity of the preparation to noradrenaline at the end of the storage period, a result fitting well with loss of uptake sites due to degeneration of nervous tissue.

As well as being excited by electrical stimulation, the nerves in the portal vein are excited by nicotine, but the muscle cells are not. Thus, in the various conditions used, nicotine can reproduce the effects of transmural stimulation. In the untreated preparation nicotine in small doses caused a relaxation and this was replaced by a contraction or biphasic effect at higher doses. In the same way, transmural stimulation at low frequencies caused relaxations, but as the frequency was increased these were replaced by contractions. The contractor effects of both transmural stimulation and of nicotine were abolished by α-adrenoceptor blockade or by adrenergic neurone blocking agents. Both these effects, therefore, were due to excitation of sympathetic nerves.

The relaxations induced either by electrical stimulation or by nicotine have been analysed in detail. In untreated preparations either a pure relaxation or after-relaxations lasting for 10–20 min were elicited by transmural stimulation. Part of these relaxations was due to activation of sympathetic nerves followed by excitation of α-adrenoceptors, for they were shortened by antagonists of α-adrenoceptors, adrenergic neurone blocking agents or by tetrodotoxin. Vasodilatation resulting from excitation of α-adrenoceptors by noradrenaline has been described (Gokhale, Gulati, Kelkar & Kelkar, 1966), but we were only able to induce pure relaxations with noradrenaline after α-adrenoceptor block. Relaxations following contractions induced by noradrenaline were occasionally seen, but the loss of tone was prolonged and recovery to the initial resting tone often took 30 min. The after-relaxation which followed a contraction induced by transmural excitation of sympathetic nerves might have been linked in some way to the contractile process. However, the fact that similar relaxations could be induced either by low frequency stimulation or by high frequency stimulation when the contraction (and some of the relaxation) had been abolished by α-adrenoceptor block suggested that it was a separate phenomenon.

In the presence of α-adrenoceptor blocking agents nicotine, like transmural stimulation, still produced a relaxation of the portal vein. These relaxations were shortened by β-adrenoceptor blocking agents, showing that in part they were due to excitation of β-adrenoceptors. Cocaine did not alter the β-adrenoceptor effects induced by electrical stimulation although relaxations elicited by noradrenaline were enhanced. This contrasts with the potentiation by cocaine of the α-adrenoceptor effects induced by electrical excitation or by noradrenaline (Hughes & Vane, 1967). The onset of the β-adrenoceptor response induced by electrical stimulation was also much slower than the onset of the α-adrenoceptor response. These observations suggest that the β-adrenoceptors are more distant from the sympathetic nerves than the α-adrenoceptors. This might also explain why the sympathetic component of the relaxation after α-adrenoceptor block was only significant at frequencies above 6–8 Hz.

When the effects of sympathetic nerve stimulation were completely abolished by adrenergic neurone blocking agents or by antagonists of both α- and β-adrenoceptors, either transmural stimulation or nicotine still induced a relaxation, showing that both procedures also excited a non-adrenergic nervous pathway. This conclusion was supported by the results in which relaxations were obtained with nicotine and transmural stimulation in preparations from rabbits pretreated with reserpine. These non-adrenergic nerves were blocked by tetrodotoxin, but higher concentrations were needed than for the sympathetic nerves. Antidromic stimulation of afferent fibres causes a pronounced and sustained vasodilatation in the cutaneous vasculature (Holton & Perry, 1951 ; Celander & Folkow, 1953). The non-adrenergic relaxation of the portal vein might have been caused by antidromic stimulation of such sensory fibres, although the duration of the relaxation in the portal vein is much shorter than that seen in the rabbit ear (Holton & Perry, 1951).

Whatever type of nervous tissue is stimulated in these conditions the nature of the transmitter remains elusive. The fact that the response can be obtained in the presence of both α- and β-adrenoceptor blocking agents and of hyoscine indicates that neither adrenergic nor cholinergic systems are involved. Since prostaglandins E_1, E_2 and $F_{2\alpha}$ cause contractions of the portal vein, it is unlikely that any of these

substances are the inhibitory transmitter. The lack of effects of dipyridamole, which potentiates some of the effects of adenosine and its derivatives (Stafford, 1966), either on the relaxation induced by stimulation or on that elicited by adenosine derivatives, make it impossible to conclude whether these substances play a part.

All the effects of nicotine (the contraction mediated by α-adrenoceptors and the relaxations mediated by β-adrenoceptors and by excitation of non-adrenergic nerves) were reduced or abolished by much lower concentrations of cocaine than were required to prevent the responses induced by electrical stimulation. This suggests that the action of nicotine is more susceptible to the membrane-stabilizing effects of cocaine than is the excitation by electrical stimulation. This interpretation also explains the antagonism of the action of nicotine by propranolol and bretylium, for these drugs have some local anaesthetic action. Burnstock, Campbell & Rand (1966) also concluded that relaxations induced by nicotine in the taenia of the guinea-pig were due to excitation of non-adrenergic inhibitory nerves. They found that bretylium markedly reduced the effects of nicotine and attributed this to a ganglion blocking effect. The antagonism of the actions of nicotine by hexamethonium does not necessarily mean that a ganglionic site is involved. There is considerable evidence that nicotine and acetylcholine can stimulate postganglionic sympathetic nerves at sites blocked by hexamethonium (Coon & Rothman, 1941; Duniér & Pernow, 1952; Kottegoda, 1953; Thompson, 1958; Burn, Leach, Rand & Thompson, 1959; Brandon & Rand, 1961; Daly & Scott, 1961). Electrophysiological experiments also show that excitation of nicotinic sites causes centripetal discharge in sympathetic C fibres (Ferry, 1963; Cabrera, Torrance & Viveros, 1966). Nicotine has been shown to cause a vasodilatation in skeletal muscle by stimulating cholinergic nerves (Hilton, 1954); however, the lack of effect of hyoscine shows that cholinergic nerves were not involved in our experiments. Nicotine and acetylcholine also excite sensory nerves (Brown & Gray, 1948; Douglas & Gray, 1953; Keele & Armstrong, 1964); such an effect might lead to the release of a vasodilator substance in the portal vein.

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