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Response to acetylcholine and nicotine of the perfused vessels of the rabbit ear

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Summary

1. The vessels of the isolated rabbit ear were perfused at 23 mbar with Krebs solution with (tonic) and without (atonic) noradrenaline ($5{\cdot}9 \times 10^{-7}$M) at selected temperatures of 20°–38° C. Peripheral resistance units (PRU) were calculated from the observed peak flow rates and alterations caused by drugs expressed as $\Delta\%$ PRU.

2. ACh is constrictor in the atonic vessel.

3. ACh is a vasodilator of the tonic vessel perfused with NA. This effect is potentiated by anticholinesterase and by denervation, unaffected by botulinum toxin and antagonized by atropine. ACh also dilates the vessel perfused with vasopressin.

4. Increasing the temperature reduces the responses to ACh but increases the effect of anticholinesterase.

5. Nicotine causes a dose dependent dilatation of the tonic vessels, reduced but not abolished by C_6, by atropine, by botulinum toxin and by denervation.

6. Nicotine causes a dose dependent constriction of the atonic vessels, abolished by C_6 and by phentolamine, reduced by denervation, but unaffected by botulinum toxin.

Introduction

The obvious accessibility of the central artery in the ear of the rabbit has made it a familiar preparation for physiological and pharmacological research since it was popularized by Dale (1914). The vessels of the isolated whole ear steadily lose tone if perfused for several hours with a saline solution. In the early stages when residual tone is present, single injections of 1–100 μg acetylcholine (ACh) cause a transient vasodilatation (Dale, 1914; Armin & Grant, 1953; Burn & Robinson, 1951; Kottegoda, 1953; Hilton, 1954; Rogers & Leaders, 1966). This action is more easily demonstrated if the tone of the vessel is maintained by stimulation of the sympathetic nerve (De La Lande & Rand, 1965; Gay, Rand & Ross, 1969) or by addition of a vasoconstrictor such as noradrenaline (NA) to the perfusate (Al Tai & Graham, 1969, 1970).

Vasodilatation by μg doses of ACh is increased by physostigmine and abolished or occasionally reversed by atropine (Burn & Dutta, 1948; Kottegoda, 1953; Burn & Rand, 1958). Larger doses of ACh (for example 5 mg: Hunt, 1918) cause vasoconstriction. This action is only demonstrable in the vessel which lacks tone (Burn & Robinson, 1951) and is enhanced by atropinization. It can be abolished by the

use of tolazoline (Burn & Dutta, 1948) or hexamethonium (C_6) (Kottegoda, 1953) or reserpine (Burn & Rand, 1958) but is present in the denervated ear vessel (Ginzel & Kottegoda, 1953). Hunt (1918) showed that ACh (2·5 μg) injected into the vessel of the rabbit ear caused vasodilatation, while 5 mg caused vasoconstriction which was not prevented by previous perfusion with nicotine. Nicotine (5–20 μg of tartrate) always causes constriction in the vessel lacking in tone (Hilton, 1954 ; Burn & Rand, 1958) an effect which is reduced by atropinization (Rogers & Leaders, 1966). It was decided to look for a nicotine induced vasodilatation in the vessel constricted by NA, which has been little used for such purposes.

The ear contains cholinesterase (Thompson & Tickner, 1953 ; Grant & Thompson, 1963) in the skin and nerves, most of it butyrylcholinesterase. The circulation through the ear in the rabbit is much altered by change of environmental temperature. It was decided, therefore, to examine the effect of temperature on the actions of acetylcholine and related drugs in the perfused ear.

Methods

The isolated rabbit ear was perfused via its central artery with gassed Krebs solution at 93 mbar (70 mmHg) pressure and controlled temperature (Page, 1944). The ear was maintained in a chamber at regulated ambient temperature. The flow in ml/min was measured directly and also by drop counter and Thorp recorder. The effect of drugs is expressed as change in an index of peripheral resistance where peripheral resistance units (PRU)=pressure in mmHg/peak flow in ml per min (1 mmHg≡1·333 mbar). If C=PRU under control conditions and R=PRU during response to a drug, $\Delta\%$ PRU= $(\frac{C-R}{C}) \times 100$ reflects the effect of the drug on the vascular bed. PRU measured during the response to a drug refers to its effect on peak flow. Statistical significance was calculated by the *t* test. The drugs examined were injected in a standard volume of 0·1 ml into the perfusion system close to the ear or dissolved in the reservoir of perfusate. The 'molar' concentrations are calculated from the proportion of base in each salt. The drugs examined were: acetylcholine chloride, atropine sulphate, echothiophate iodide, hexamethonium bromide, nicotine tartrate, noradrenaline acid tartrate, physostigmine salicylate, phentolamine hydrochloride, vasopressin injection B.P. and botulinum A toxin.

Two preparations were used: (1) the atonic preparation obtained by perfusing for 3 h with Krebs solution ; (2) the tonic preparation obtained by adding noradrenaline (NA) at a concentration of $5{\cdot}9 \times 10^{-7}$M to the perfusate and waiting until tone was established (about 30 min).

In six preparations tone was induced by perfusion with vasopressin 2 U/l. as an alternative to NA. Six ears from three rabbits were perfused for 1·5 h with botulinum A toxin in a final concentration of 2×10^{-6} g/ml in Krebs solution at 23° C, then NA was added to $5{\cdot}9 \times 10^{-7}$M and ACh and nicotine tested as before. In six rabbits anaesthetized with pentobarbitone (30 mg/kg) and ether, superior cervical ganglionectomy, cutting the great auricular nerve, and careful stripping of the central artery was performed on one side. Both ears were perfused 3–4 weeks later.

Results

When ACh is injected into the atonic preparation it causes a dilatation in doses of 0·1 ml of 10^{-8}M to 10^{-4}M. Since the vessel is atonic this response is small. Higher

concentrations (10^{-3}M) cause a constriction. In the tonic preparation it gives rise to a dose dependent dilatation from 10^{-12}M to 10^{-4}M which is illustrated in Fig. 1, panel A at 38° C. This response is potentiated by denervation (see Fig. 1 panel B) at 38° C but not at 20° C. It is also potentiated by anticholinesterases at 38° C and 20° C but to a greater extent at the higher temperature. The presence of botulinum A toxin in the reservoir fluid does not alter the dilator dose-response

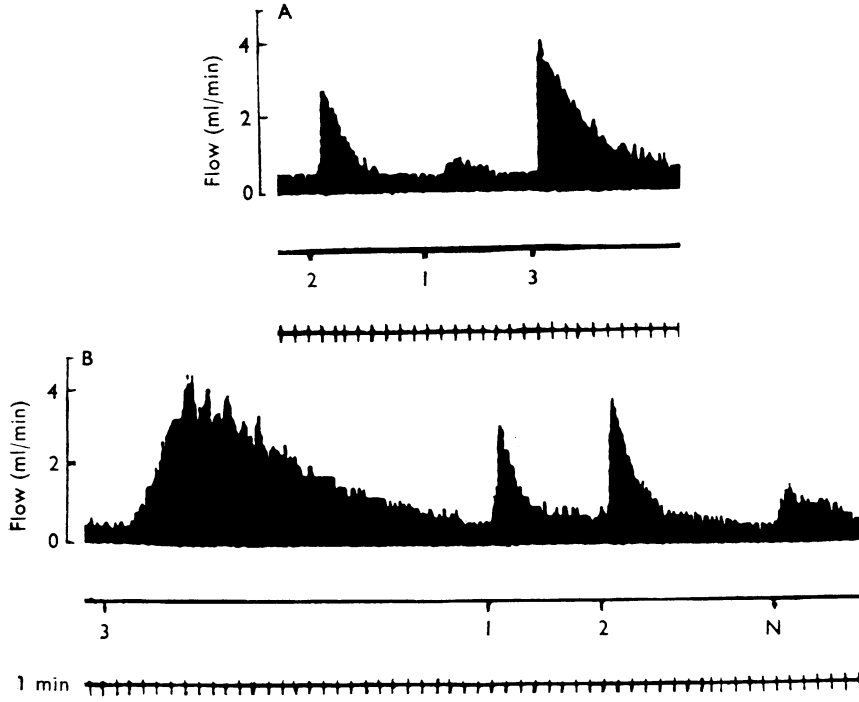

FIG. 1. Responses to ACh of the tonic preparation of perfused rabbit ear; injections of 0·1 ml at 38° C. Ordinate, flow in ml/minute. Dilatation thus ↑. Panel A, $1=10^{-12}$M; $2=10^{-8}$M; $3=10^{-4}$M. Panel B, the same after denervation. Nicotine 10^{-4}M (N) causes a transient dilatation.

TABLE 1. *Effect of injection of ACh* (10^{-12}M *and* 10^{-4}M) *on* Δ% *PRU with temperature and drugs on the perfused vessels of the rabbit ear*

Perfusate	Low conc. ACh (10^{-12}M) Δ% PRU	% Difference	High conc. ACh (10^{-4}M) Δ% PRU	% Difference
Temperature 20° C				
Krebs+NA (5·92×10^{-7}M)	52		84	
+Physostigmine (10^{-6}M)	60	+15·4	86	+2·4
+Echothiophate (10^{-6}M)	59	+13·5	86	+2·4
Temperature 38° C				
Krebs+NA	30		68	
+Physostigmine (10^{-6}M)	51	+70	80	+17·6
+Echothiophate (10^{-6}M)	53	+76·6	81	+19·1

$P<0.001$ in all cases. (Student's t test.)

curve to ACh but it is antagonized by atropine. Increasing the temperature of the ear and perfusate from 20° C to 38° C reduces the effectiveness of ACh (see Table 1). The slope of the dose-response relation to ACh is unaltered by change of

TABLE 2. *Effect of temperature on responses (Δ% PRU) to low and high concentrations of ACh in tonic preparations from the normal and denervated ears with and without anticholinesterases*

Temperature	Perfusate Krebs+NA ($5{\cdot}9\times10^{-7}$M)	Low conc. 10^{-12}M ACh	% Difference	High conc. 10^{-4}M ACh	% Difference	*P*
Normal at 38°C	Krebs+NA	30		68		
Denervated ,,	Krebs+NA	53	+77	80	+18	<0·001
Normal at 38°C	+Physostigmine (10^{-6}M)	51	+70	80	+18	<0·001
Denervated ,,	+Physostigmine	53	+77	80	+18	
Normal at 20°C	Krebs+NA	52	+73	84	+24	<0·001
Denervated ,,	Krebs+NA	52		84	+24	
Normal at 20°C	+Physostigmine	60	+100	86	+27	<0·001
Denervated ,,	+Physostigmine	52	+73	84	+24	

All differences are derived from control (normal at 38°C).

TABLE 3. *Dilator responses to ACh (Δ% PRU)*±S.E. *of the mean*

Mixtures	ACh (10^{-12}M)	ACh (10^{-10}M)	ACh (10^{-8}M)	ACh (10^{-6}M)	ACh (10^{-4}M)
Krebs+NA ($5{\cdot}9\times10^{-7}$M)	52±1·9	62±1·8	70±1·9	77±1·2	84±1·0
Krebs+NA+atropine (10^{-12}M)	46±1·6	53±1·4	61±1·4	68±2·2	74±1·9
Krebs+NA+atropine (10^{-8}M)	16±2·2	26±3·1	38±1·9	52±2·0	61±1·6

In all cases twenty-four experiments were performed (twelve animals). *P* between each pair <0·001. (Student's *t* test.)

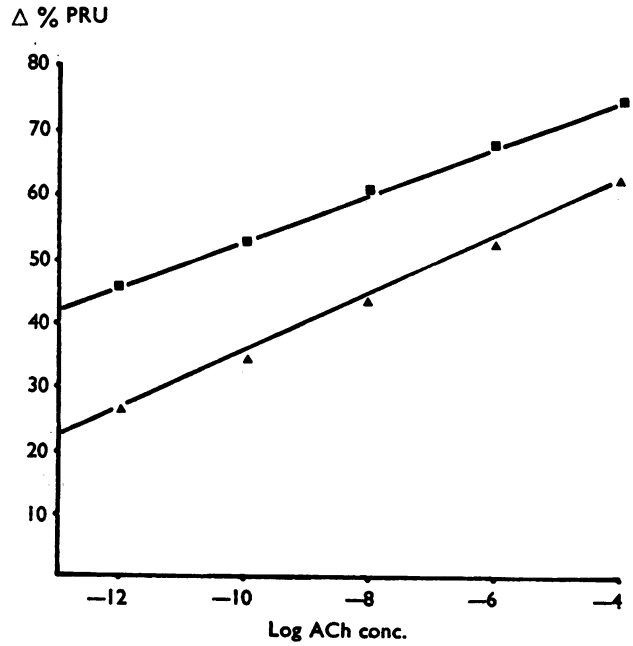

FIG. 2. Perfused tonic ear vessels of the rabbit. Log concentration-response curves to ACh in the presence of atropine (10^{-12}M). Atropine (10^{-12}M) is less effective as an antagonist to ACh (Δ% PRU) in the perfused tonic ear vessels of the rabbit over the whole range of doses at 20° C (upper curve) than it is at 38° C (lower curve).

temperature. Perfusing with physostigmine (10^{-6}M in the reservoir) or echothiophate (10^{-6}M) potentiates the dilator response to ACh. This effect is more marked when the concentration of injected ACh is low (10^{-12}M). The potentiation is also temperature dependent, being less at 20° C than at 38° C (see Table 1).

The potentiating effect of physostigmine, which is quite marked in the innervated vessel, is abolished by denervation (see Table 2). The dilator effect of ACh is present in the vessel constricted by vasopressin but is not so marked as in the vessel perfused with NA. This partial failure may be solely quantitative, that is, the spasm induced by vasopressin (2 U/l.) is more intense than that caused by NA, $5{\cdot}9 \times 10^{-7}$M, and accordingly more difficult to overcome. Perfusing with atropine reduces the dilator effect of ACh and this antagonism, being a competitive one, is more marked on low concentrations of ACh (10^{-10}M) than on high (10^{-4}M) and is more marked with high concentrations of atropine than low (see Table 3). It is also temperature dependent, being less effective in a fixed concentration of 10^{-12}M atropine over the range of ACh 10^{-12}M–10^{-4}M at 20° C than 38° C (see Fig. 2).

Nicotine, like ACh has an ambivalent effect, according to the tone of the vessel. If this is high (NA perfusion) nicotine causes dilatation. This action is dose dependent from 10^{-10}M to 10^{-4}M (Fig. 3) and is about half of that produced under identical circumstances by equimolar amounts of ACh (Table 4). Atropine (10^{-6}M) which abolishes the dilator response to equimolar ACh reduces markedly but does not abolish this effect of equimolar nicotine. Hexamethonium (10^{-6}M) also markedly reduces but does not abolish this response. Botulinum toxin abolishes the dilator action of nicotine, 10^{-10}M–10^{-8}M, and significantly reduces the dilator responses to nicotine, 10^{-6}M–10^{-4}M, (Table 5). Denervation has a similar effect of equal degree.

Nicotine constricts the atonic vessel. Botulinum toxin does not modify this but denervation abolishes the effect of nicotine, 10^{-4}M, and greatly reduces that of 10^{-3}M. If the tone is low or absent nicotine causes vasoconstriction but it requires a higher

TABLE 4. *Comparison between ACh and nicotine dose-response (Δ% PRU) in tonic preparations at 23°C*

	Responses in Δ% PRU			
Molar conc.	10^{-11}M ± S.E.	10^{-8}M ± S.E.	10^{-6}M ± S.E.	10^{-4}M ± S.E.
Acetylcholine	62 ± 1·8	70 ± 1·9	77 ± 1·2	84 ± 1
Nicotine	26 ± 2·2	38 ± 1·6	47 ± 2	55 ± 2
% Difference from ACh	−58	−46	−39	−34

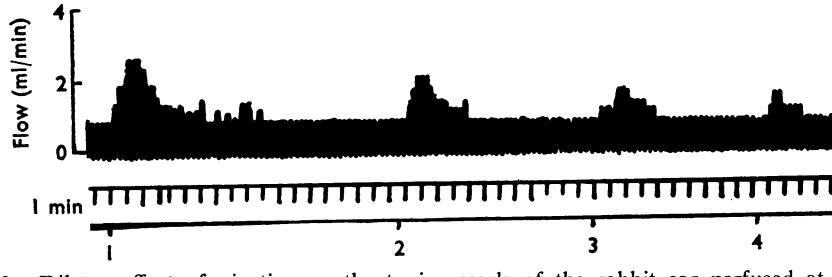

FIG. 3. Dilator effect of nicotine on the tonic vessels of the rabbit ear perfused at 23° C. 0·1 ml injections; 1 = 10^{-4}M; 2 = 10^{-6}M; 3 = 10^{-8}M; 4 = 10^{-10}M.

concentration (10^{-4}M and 10^{-3}M) than that needed to cause vasodilatation in the vessel with high tone. The constrictor response is dose related over a narrow range and is abolished by C_6 10^{-6}M and by phentolamine $3{\cdot}5 \times 10^{-6}$M (Fig. 4).

Discussion

The dilator (muscarinic) response to ACh (0·1 ml of 10^{-12}M–10^{-4}M) in the perfused vessel with high tone is dose related and is relatively greater at 20° C than at 38° C. This implies that the chilled tissue is more sensitive to ACh than at normal temperature or is equally responsive but more ACh reaches the cholinoceptive sites. This could be because of a reduction in the rate of destruction by cholinesterase which is temperature sensitive. When this system is inhibited by physostigmine or echothiophate the potency of the anticholinesterase in potentiating responses to ACh is relatively less at 20° C and greater at 38° C and less effective the higher the concentration of ACh as substrate. This implies that the increased sensitivity to ACh at low temperatures reflects a decrease in destruction rather than an increase in other factors such as affinity or reactivity of the muscle. The dilator action of ACh is exerted on the vessel perfused with vasopressin so it cannot be due to NA antagonism as postulated by Rand & Varma (1970). Denervation and botulinum toxin do not affect it so it is a direct action on the smooth muscle. Holton & Rand (1962) noted an increased sensitivity to ACh dilatation after decentralization of the cervical ganglion, and Armin, Grant, Thompson & Tickner (1953) reported that denervation caused a loss of cholinesterase from the vessel while monoamine oxidase was retained.

The antagonism exerted by atropine is likewise relatively greater when the concentration of agonist ACh is low but the temperature relation is the opposite to that of physostigmine. This suggests a temperature sensitive reaction in which the sub-

TABLE 5. *Effect of denervation and of botulinum A toxin on the dilator responses of the tonic vessels of the perfused rabbit ear to nicotine*

	Responses in Δ% PRU	
Molar concentration	10^{-6}M±S.E.	10^{-4}M±S.E.
Innervated control	47±2	55±2
Denervated	23±1·7	39±1·7
% Difference	−51 $P<0{\cdot}001$	−29 $P<0{\cdot}001$
Botulinum toxin	22±1·6	35±1·5
% Difference	−53 $P<0{\cdot}001$	−36 $P<0{\cdot}001$

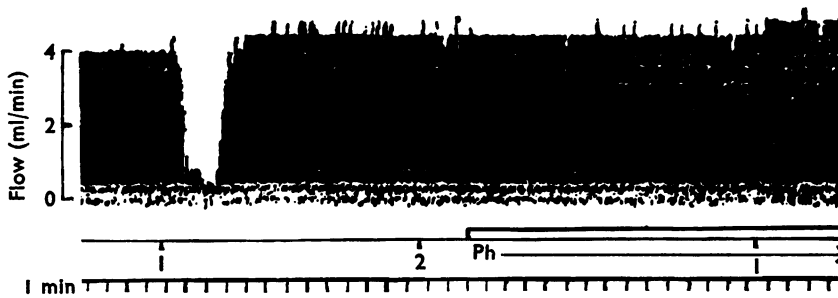

FIG. 4. Constrictor effect of injections of 0·1 ml nicotine solution on the atonic vessels of the rabbit ear at 23° C is abolished by phentolamine, $3{\cdot}5 \times 10^{-6}$M, (Ph) whereas the after dilatation is not. 1=nicotine 10^{-3}M ; 2=10^{-4}M.

strate is atropine and the enzyme presumably atropinase (Bernheim & Bernheim, 1938 ; Hobbiger & Lessin, 1955) and a predominance of clearance of atropine over clearance of ACh in the final result. It is unlikely on thermodynamic grounds that the affinity of atropine for these receptors is higher at the lower temperature. Vasoconstriction by nicotine is well documented but there has been doubt about vasodilatation. Now there is a clear demonstration of a dose dependent effect, reduced by botulinum toxin and denervation. The greater part of this action must be on cholinergic vasodilator nerves, a residual small part on another mechanism, probably the muscle. Studies at the fine structural level to identify more precisely the nature of the innervation of the vascular smooth muscle are needed.

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