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POTENTIATION OF THE CONTRACTILE RESPONSE TO ACETYLCHOLINE IN AORTIC STRIPS BY LOW CONCENTRATIONS OF VASCULAR CONTRACTILE AGONISTS

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1 The potentiating effect of a low concentration of various vascular contractile agonists on the response of rabbit aortic strips to acetylcholine was examined.

2 A marked parallel displacement to the left (potentiation; not additive effect) of the dose-response curve for acetylcholine was observed when 5-hydroxytryptamine (5-HT), noradrenaline, histamine, angiotensin II or KCl were added at a concentration that caused only slight contraction (0.2 to 0.3 g) in the strips. This leftward displacement (potentiation) of the dose-response curve by these agonists was reversed to the control response curve by the respective blocking agents for each agonist, although the blocking agents themselves did not shift the acetylcholine dose-response curve to the right. Atropine produced a rightward displacement of the dose-response curve for acetylcholine.

3 The addition of an extremely low concentration of 5-HT or KCl which did not cause contraction, also produced a potentiation.

4 The order of potency of the agonists in potentiating the acetylcholine-induced contraction was KCl > 5-HT $\geqq$ histamine $\geqq$ angiotensin II > noradrenaline.

5 The contractile response of aortic strips to carbamylcholine was also potentiated by these agonists.

6 The results suggest that the potentiation by these agonists was not mediated through muscarinic receptors but through the respective receptors for each agonist.

Introduction

The action of cholinomimetic drugs on isolated aortic strips has not been described in detail. A few papers are cited which deal with the vascular contractile response to acetylcholine, but any discussion of the effects of acetylcholine on rabbit aortic strips is noticeably absent (Furchgott & Bhadrakom, 1953; Hudgins & Fleming, 1966; Tayler & Green, 1971). Furchgott & Bhadrakom (1953) and Tayler & Green (1971) pointed out that the maximum contractile response of the aortic strip to acetylcholine is considerably less than that to sympathomimetics. Hudgins & Fleming (1966) found that aortic strips from rabbits pretreated with reserpine exhibit what they term a 'relatively nonspecific supersensitivity', i.e., the strips were supersensitive to (−)-noradrenaline, acetylcholine and KCl but not to histamine, 5-hydroxytryptamine (5-HT) and angiotensin amide. De la Lande, Cannell & Waterson (1966) documented the ability of 5-HT to potentiate responses to a number of contractile agonists such as noradrenaline, adrenaline, histamine and angiotensin the perfused rabbit ear artery. However, the potentiation of the response to acetylcholine by these contractile agents has never been described. In the present study, evidence is presented that the contractile response of the aortic strip to acetylcholine is potentiated in the presence of a low concentration of other contractile agonists.

Methods

Albino rabbits of either sex weighing 2.2 to 2.6 kg were killed by bleeding from the carotid artery, and the thoracic aorta (3.0 to 4.5 mm o.d.) between the heart and diaphragm was quickly excised. After removal of excess fat and adventitial connective tissue, the aorta was cut at an angle of approximately 45° to the longitudinal axis into spiral strips, 2.5 mm in width and 30 mm in length, according to the method of Lewis & Koessler (1927), or Furchgott & Bhadrakom (1953). The spiral strip was fixed vertically between hooks in a water jacketed (37 ± 0.5°C) tissue bath containing 40 ml of modified Krebs-Henseleit solution (pH 7.4). The composition of the bathing

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solution used was as follows (mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and dextrose 10.0. The tissue bath solutions were maintained at 37 ± 0.5°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The upper end of the strip was connected to the lever of a force-displacement transducer (SB-1T, Nihon Kohden Kogyo, Co., Tokyo, Japan) by a silk thread. An initial resting tension of 2 g was applied to the aortic strips. Before the experiments were started, strips were allowed to equilibrate for 1 h in the bathing solution. During the equilibration period, the strip relaxed and the resting tension was always readjusted to 2 g; the solution was replaced every 15 min.

After the hour of equilibration at a resting tension of 2 g, a submaximally effective concentration of KCl (40 mM) was given two or three times until successive responses remained constant. Cumulative dose-response curves for a contractile agonist were obtained by a stepwise increase in the concentration of the agonist as soon as a steady response was obtained to the preceding dose. The concentration of the agonist in the bath was increased by a factor of 3 or 10 until the maximum response was obtained (van Rossum, 1963). Concentrated stock solutions of drugs were added directly to the bathing solution in a volume of 0.4 ml to give the final concentrations desired. Effects of various blocking agents such as atropine, phentolamine, methysergide and diphenhydramine on the contractile response to acetylcholine were determined.

Whenever an ED_{50} value was determined, responses to acetylcholine were calculated as a percentage of the maximum contraction obtained with that agonist. The ED_{50} value was obtained visually from a graph of percent contraction plotted against log concentration of the agonist and expressed as the negative logarithm (pD_2 value). Results shown in the text, tables and figures are expressed as the mean value ± s.e. Comparison of the results was by Student's t test, paired t test or analysis of variance (Finney, 1964). Statistical significance was assumed when $P < 0.05$.

Determination of apparent dissociation constants (K_B) and pA_2 values

Values for K_B, the dissociation constant of the receptor-inhibitor complex, were calculated by the method of Furchgott (1967) from the following equation:

$$K_B = \frac{[B]}{\text{dose-ratio} - 1}.$$

Dose-ratio refers to the concentration of agonist required to elicit 50% of the maximum response (ED_{50}) in the presence of a concentration [B] of the antagonist, divided by the ED_{50} in the absence of the antagonist. Dose-response curves were determined simultaneously on four individual aortic strips. Three of the strips were subjected to different concentrations of atropine; the fourth strip was a control, serving as an indicator of changes in tissue sensitivity during the course of the experiment. If such changes were noted, dose-ratios were corrected accordingly. The three values were then averaged to obtain a K_B for that particular tissue. This formula can only be used when the agonist and the antagonist are competitive with one another. The K_B values were independent of the concentration of the antagonist, which is one indication that such a relationship existed. Also, the slopes of the plots of log (dose-ratio − 1) vs −log[antagonist] were close to the theoretical value of −1 (Table 2) (Arunlakshana & Schild, 1959). These values and their corresponding pA_2 values (negative logarithm of the molar concentration of antagonist which reduces the effect of an agonist to that of half the dose; Arunlakshana & Schild, 1959) were determined by regression analysis.

Drugs and chemicals

These included acetylcholine chloride (Wako), serotonin (5-hydroxytryptamine) creatinine sulphate (Merck), (−)-noradrenaline bitartrate (Wako) histamine diphosphate (Nakarai), angiotensin II (Protein Research Foundation), carbamylcholine chloride (Sigma), atropine sulphate (Katayama), phentolamine mesylate (Regitine mesylate, Ciba), methysergide dimaleate and diphenhydramine hydrochloride (Tokyo Kasei). All drugs were prepared daily in Krebs-Henseleit solution and kept on ice during the course of the experiment.

Results

Potentiation of vascular contractile response to acetylcholine by a low concentration of various contractile agonists

The addition of acetylcholine in concentrations ranging from 1×10^{-6} M to 3×10^{-3} M elicited a dose-dependent contraction in isolated aortic strips of the rabbit. When the dose-response curve for acetylcholine was determined in the presence of a low concentration of such agonists as 5-HT, noradrenaline, histamine, angiotensin II or KCl which caused only slight contraction (0.2 to 0.3 g) in the strips, there was a marked increase in the contractile response to acetylcholine. In order to determine whether the effect of these contractile agonists on acetylcholine-induced contraction was a 'potentiation' or an 'additive effect', studies were carried out as follows. Figure 1 shows the control dose-response curve determined in the

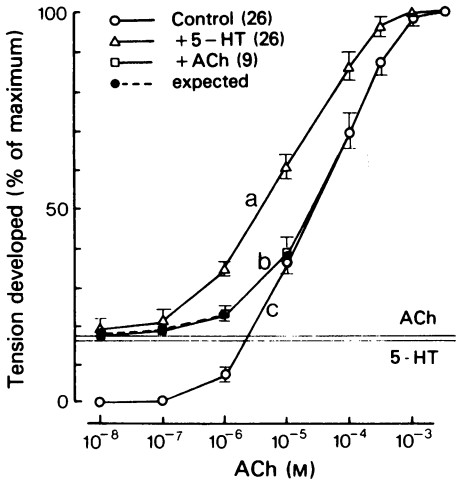

Figure 1 Potentiating effect of 5-hydroxytryptamine (5-HT) on the response to acetylcholine (ACh). Curve (c) (○), control curve for acetylcholine in the absence of 5-HT. Curve (a) (△), dose-response curve for acetylcholine determined in the presence of 5-HT 1.4×10^{-8} M; 5-HT itself caused an increase in tension of $16.4 \pm 1.9\%$ (lower horizontal line). Curve (b) (□), effect of acetylcholine in the presence of ACh 2.3×10^{-6} M which itself caused an increase in tension of $17.8 \pm 4.4\%$ (upper horizontal line). Broken line (---●---), 'expected' dose-response curve; for details see text. In each dose-response curve maximum contractile tension developed by ACh 3×10^{-3} M was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used.

absence of any 5-HT (curve c). A second aortic strip was exposed to 5-HT 1.4×10^{-8} M until the small response to this agonist had reached a plateau (lower horizontal line of Figure 1); thereupon, acetylcholine was administered, and the result is shown as curve (a) in Figure 1. The difference between curve (c) (control) and (a) may be due to potentiation of the response to acetylcholine by 5-HT but it may also be due to the addition of the effects of a constant concentration of 5-HT to those of the increasing amounts of acetylcholine. In order to distinguish between these two possibilities, small amounts of acetylcholine were administered to a third aortic strip until the small response to acetylcholine was nearly equal to that previously observed with 5-HT 1.4×10^{-8} M; approx. 2.3×10^{-6} M of acetylcholine was required for this (upper horizontal line of Figure 1). When the small response to acetylcholine had reached a plateau, successive increasing amounts of acetylcholine were added to the bath as in the preceding experiments. The resulting dose-response curve (curve b of Figure 1) converged with the control curve (c) and was identical with it at the higher concentrations. As pointed out earlier (Trendelenburg, 1962; Langer & Trendelenburg, 1966; Draskóczy & Trendelenburg, 1968) such a convergence of dose-response curves is typical of an additive effect.

Further evidence for this view was obtained as follows. From the effects of the initial treatment with acetylcholine 2.3×10^{-6} M (upper horizontal line of Figure 1) and from the control curve (c), it is possible to calculate the dose-response curve that must be expected from the addition of the effect of a constant concentration of 5-HT to those of the increasing amounts of acetylcholine. This is simply done by plotting responses to 2.3 + 0.01, 2.3 + 0.1, 2.3 + 1, 2.3 + 10, 2.3 + 100, 2.3 + 300, 2.3 + 1000, 2.3 + 3000 μM of acetylcholine. The broken curve of Figure 1 is the result; it shows that the 'expected dose-response curve' is identical with curve (b); however, it is clearly different from the curve obtained in the presence of 5-HT 1.4×10^{-8} M (curve a). This may be taken as evidence that 5-HT causes supersensitivity to acetylcholine.

The dose-response curve for acetylcholine was also shifted to the left when the response to acetylcholine was measured in the presence of noradrenaline (5.1×10^{-9} M), histamine (3.1×10^{-7} M), angiotensin II (1.0×10^{-10} M) or KCl (8.5 mM). When the small responses to these agonists had reached a plateau, successive increasing amounts of acetylcholine were added as in the preceding experiments. A comparison of the results and the 'expected dose-response curve' showed that the contractile response to acetylcholine was also potentiated by the addition of noradrenaline, histamine, angiotensin II or KCl.

Table 1 summarizes the effects of these contractile agonists on the pD_2 value and the maximum contractile tension of acetylcholine. It is evident that 5-HT, noradrenaline, histamine, angiotensin II and KCl produce potentiation of the response to acetylcholine but acetylcholine itself causes no potentiation. Whereas the increases in sensitivity induced by 5-HT, histamine and angiotensin II were similar, the increase in sensitivity induced by noradrenaline was a little smaller, and the increase in sensitivity by KCl was much greater in magnitude.

The effects of 5-HT 7×10^{-9} M, or of KCl 5 mM, which did not cause contraction, were analyzed in another series of experiments. On 16 aortic strips three dose-response curves for acetylcholine from a single preparation were determined with an interval of 60 min between each determination. In one group (eight aortic strips), pD_2 value of the third curve was decreased by 0.02 ± 0.003 log units with respect to the second curve. In the second group (eight aortic strips), the third dose-response curve was determined in the presence of 5-HT 7×10^{-9} M (which itself had no effect on contractile tension). In this group, the third pD_2 value was increased by 0.23 ± 0.04 log

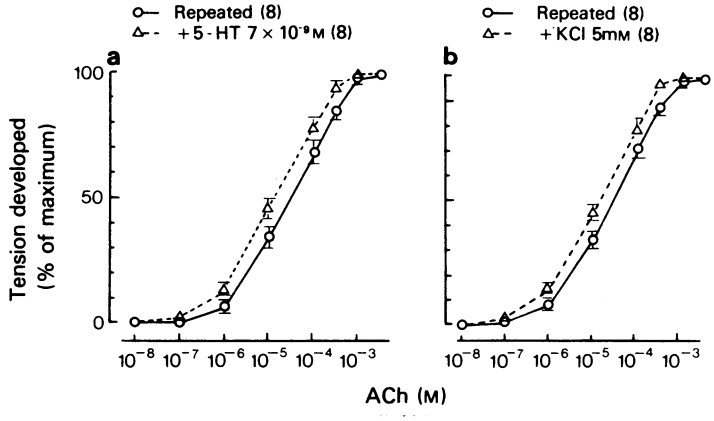

Figure 2 Effect of a low concentration of 5-hydroxytryptamine (5-HT, a) or KCl (b) on the dose-response curve for acetylcholine (ACh). Three cumulative dose-response curves for ACh were obtained from a single preparation. 5-HT 7×10^{-9} M or KCl at a concentration of 5 mM, which did not cause a contraction, was added 10 min before the third trial (Δ). Repeated dose-response curve was determined in the absence of 5-HT or KCl at the third trial (O); for details see text. In each dose-repsonse curve, maximum contractile tension developed by ACh 3×10^{-3} M was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used.

units with respect to the second. This represents a small but highly significant ($P < 0.01$) potentiating effect of 7×10^{-9} M 5-HT (Figure 2a). The addition of 5 mM KCl also produced a potentiation of acetylcholine (Figure 2b).

Potentiation of the contractile response to carbamylcholine

It is well known that acetylcholine is inactivated by acetylcholinesterase at the neuromuscular junction during neurotransmission. If the activity of acetylcholinesterase was inhibited by the administration of these contractile agonists, the contractile response to acetylcholine might be altered. We, therefore, examined the potentiating effect of these contractile agonists on the response to carbamylcholine instead of acetylcholine. Carbamylcholine in concentrations ranging from 1×10^{-6} M to 3×10^{-3} M elicited a dose-dependent contraction in aortic strips. When the cumulative dose-response curve for carbamylcholine was determined after the small response to 5-HT, noradrenaline, histamine, angiotensin II or KCl had reached a plateau, the response to carbamylcholine was significantly potentiated by these agonists. The potentiating effects of 5-HT and KCl on the response to carbamylcholine are shown in Figure 3.

Table 1 Effects of several contractile agonists on the pD_2 value and maximum response to acetylcholine in aortic strips of the rabbit

Agonist[a] *added*	*Concentration* (M)	n	*Acetylcholine*[a] pD_2 *value*[b]	*Maximum tension*[c] (mg)
None (control)		26	4.55 ± 0.09	1259 ± 175
5-Hydroxytryptamine	1.4×10^{-8}	26	5.16 ± 0.08***	2234 ± 203**
Noradrenaline	5.1×10^{-9}	13	4.98 ± 0.11*	1862 ± 258*
Histamine	3.1×10^{-7}	14	5.11 ± 0.13**	1898 ± 314*
Angiotensin II	1.0×10^{-10}	13	5.08 ± 0.15*	2157 ± 318**
KCl	8.5×10^{-3}	16	5.42 ± 0.09***	2867 ± 351***
Acetylcholine	2.3×10^{-6}	9	4.56 ± 0.10	1259 ± 320

[a] Cumulative dose-response curve for acetylcholine was determined in the presence of contractile agonist.
[b] The ED_{50} value was obtained visually from a plot of % contraction vs log concentration of acetylcholine and expressed as the negative logarithm (pD_2 value).
[c] Maximum contractile tension was attained at acetylcholine 3×10^{-3} M.
n indicates the number of preparations used; data are expressed as mean ± s.e. mean.
Significantly different from control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

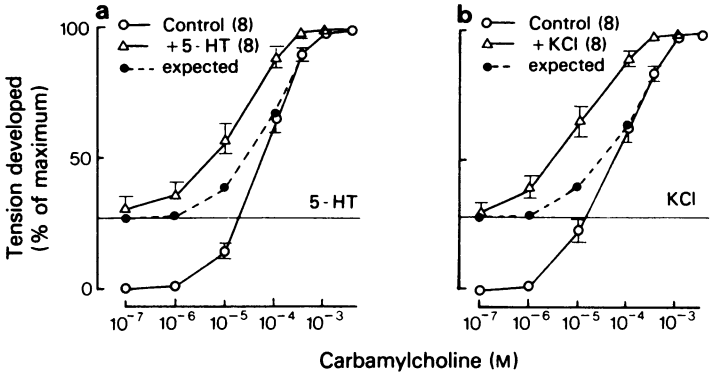

Figure 3 Potentiating effect of 5-hydroxytryptamine (5-HT, a) and KCl (b) on the response to carbamylcholine. (△): Potentiated dose-response curve for carbamylcholine determined in the presence of 5-HT 1.4×10^{-8} M; 5-HT itself caused an increase in contractile tension of 27.1 ± 5.5% (a), or in the presence of 8.5 mM of KCl; the KCl itself caused an increase in contractile tension of 28.4 ± 4.3% (b). Control (○) and 'expected' (----●----) dose-response curves shown in the figure are same as in Figure 1. In each dose-response curve, maximum contractile tension developed by carbamylcholine 3×10^{-3} M was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used.

Comparison of the apparent dissociation constant (K_B) *and* pA_2 *value of atropine in the presence and the absence of 5-hydroxytryptamine*

The potentiation by 5-HT of the contractile response to acetylcholine is exhibited as a parallel leftward displacement of the dose-response curve. Such a displacement could result from an increase in the affinity of the muscarinic receptor. Atropine, a competitive muscarinic receptor blocking agent, theoretically competes with acetylcholine for a common site on the receptor macromolecule. If so, then a change in the affinity of the receptor for the agonist might well be reflected in an alteration of the K_B and pA_2 values for atropine. Table 2 indicates that no such change occurred; K_B and pA_2 values of the aortic strips treated with 5-HT were identical to those of control strips. Furthermore, slopes of the plots of log (dose-ratio − 1) vs −log[antagonist] were close to the theoretical value of −1 (Table 2) (Arunlakshana & Schild, 1959), indicating that atropine was competitive with acetylcholine in the presence and the absence of 5-HT.

Block of potentiation by specific agonist inhibitors

To determine whether the potentiating effects of these contractile agonists were caused through the respective receptors of each agonist or not, we examined the effect of methysergide, phentolamine and diphenhydramine on the potentiated responses to acetylcholine produced by 5-HT, noradrenaline and histamine, respectively. The respective blocking agents of each agonist inhibited specifically each agonist-induced potentiation (Figure 4). It would appear therefore that the potentiation of the response to acetylcholine by

Table 2 K_B and pA_2 values for atropine in the presence and the absence of 5-hydroxytryptamine (5-HT)

Agonist/Antagonist	*Conditions*	K_B[a]	pA_2[b]	*Slope*[c]
		$\times 10^{-10}$ M		
Acetylcholine/Atropine	+5-HT[d]	7.9 ± 1.2	9.10	−0.98
	−5-HT	7.0 ± 0.9	9.16	−0.96

[a] Values for K_B, the dissociation constant of the receptor-inhibitor complex were calculated by the method of Furchgott (1967); for details see Methods. Data are expressed as mean ± s.e. mean of 8 determinations.
[b] Negative logarithm of the molar concentration of antagonist which reduced the effect of acetylcholine to that of half the dose (pA_2: Arunlakshana & Schild, 1959).
[c] Slope of the plot of log (dose-ratio − 1) vs − log[antagonist].
[d] Cumulative dose-response curve for acetylcholine was determined in the presence of 5-HT 1.4×10^{-8} M.

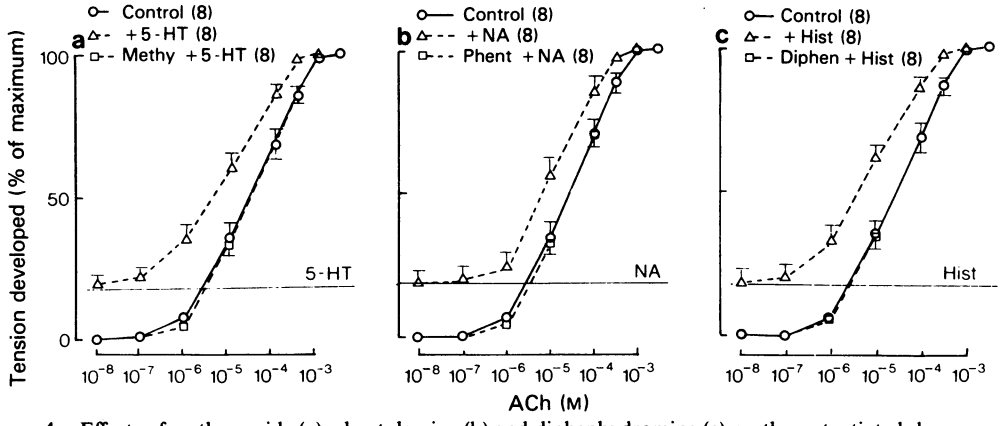

Figure 4. Effects of methysergide (a), phentolamine (b) and diphenhydromine (c) on the potentiated dose-response curve for acetylcholine (ACh) determined in the presence of 5-hydroxytryptamine (5-HT, a), noradrenaline (NA, b) and histamine (Hist, c), respectively. Methysergide (2×10^{-7} M), phentolamine (2×10^{-7} M) or diphenhydramine (1×10^{-7} M) was added 20 min before the administration of 5-HT, noradrenaline or histamine. In each dose-response curve, maximum contractile tension developed by ACh 3×10^{-3} M was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used.

these agonists was mediated through the respective receptors of each agonist rather than through muscarinic ones.

Figure 5 shows that the dose-response curve for acetylcholine in the presence of 5-HT was not attenuated by phentolamine 1×10^{-6} M suggesting that the acetylcholine-induced contraction is not mediated by the release of endogenous noradrenaline or the direct stimulation of α-adrenoceptors.

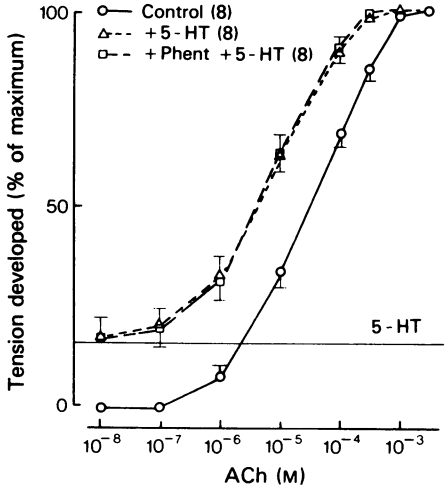

Figure 5 Effect of phentolamine (Phent) on the potentiated dose-response curve for acetylcholine (ACh) determined in the presence of 5-hydroxytryptamine (5-HT). Phentolamine 2×10^{-7} M was added 20 min before 5-HT. In each dose-response curve, maximum contractile tension developed by ACh 3×10^{-3} M was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used.

Discussion

There is much evidence for a contractile effect of acetylcholine on perfused placental vessels (von Euler, 1938), helically cut aortic strips (Furchgott & Bhadrakom, 1953; Hudgins & Fleming, 1966; Tayler & Green, 1971), umbilical arterial and venous strips (Somlyo, Woo & Somlyo, 1965; Altura, Malaviya, Reich & Orkin, 1972; Nair & Dyer, 1974), pulmonary vascular (Nuki, 1954) and coeliac venous beds (Fleisch, 1931). It is reasonable to assume that the contractile response to acetylcholine is mediated through the muscarinic receptors of cellular plasma membranes. 5-HT, noradrenaline and histamine act directly on the effector cells following interaction with their respective receptors located particularly in cellular plasma membranes (von Euler, 1946; Alhquist, 1948; Gaddum & Hameed, 1954; Vane, 1957; Paton & Vane, 1963). It has been demonstrated that KCl-induced vascular contraction, which is expected to result in an increased permeability of membranes for calcium and an increased mobilization of membrane-bound and intracellularly stored calcium, follows depolarization of cell membranes (Su & Bevan, 1964; Axelsson, Wohlström, Johanson & Jonsson, 1967). Acetylcholine, which is exogenously administered probably interferes with the muscarinic receptor of the cell membrane, and changes the ionic permeability of the membrane, thus permitting the entry of calcium ions

from the extracellular fluid (Edman & Schild, 1962; von Hagen & Hurwitz, 1967; Somlyo & Somlyo, 1968).

The acetylcholine-induced contractile response of the thoracic aorta was significantly potentiated in the presence of a low concentration of another contractile agonist such as 5-HT, noradrenaline, histamine, angiotensin II or KCl. Before any 'potentiation' or 'enhancement of maximum response' is considered, 'additive effect' must be excluded. Such 'additive effect' has been discussed in detail by Trendelenburg (1963): examples of how 'potentiation' is distinguished from 'additive effect' and of how an 'additive effect' is demonstrated to be such have been provided by Draskóczy & Trendelenburg (1968). From these examples, it is clear that 'potentiation' means a parallel displacement of the dose-response curve to the left, while only 'enhancement of maximum response' cannot be called 'potentiation'. The dose-response curve of the thoracic aorta for acetylcholine was shifted to the left by a low concentration of contractile agonist, such as 5-HT, noradrenaline, histamine, angiotensin II and KCl.

The potentiated contractile response to acetylcholine in the presence of all agonists tested was suppressed by atropine. On the other hand, methysergide, a specific blocking agent for 5-HT, also suppressed the potentiated response to acetylcholine in the presence of 5-HT and did not affect other agonist-induced potentiation of the response to acetylcholine. The potentiation of the response to acetylcholine by these contractile agonists was not mediated through muscarinic receptors but through the respective receptors for each agonist, because the respective blockers of each agonist inhibited specifically each agonist-produced potentiation. It appears more likely that vascular contractile agonists such as 5-HT, noradrenaline, histamine, angiotensin II and KCl sensitize the smooth muscle membrane to excitation by acetylcholine.

The maximum contractile response of aortic strips to acetylcholine itself is quite low compared to that seen with noradrenaline. Furchgott & Bhadrakom (1953), and Tayler & Green (1971) also pointed out that the maximum response of the aortic strip to acetylcholine is considerably less than that to sympathomimetics. Interestingly, it was shown in the latter paper, that pretreatment with reserpine produced a potentiation with an increase in the maximum response to acetylcholine. In the present study, various vascular contractile agonists also produced a potentiation with an increase in the maximum response to acetylcholine which was still less than that to noradrenaline.

The antagonistic effect of atropine on acetylcholine-induced contraction of aortic strips observed in the present study suggests a direct action of acetylcholine on muscarinic receptors. Phentolamine at a concentration of 1×10^{-6} M (a sufficient dose to shift the dose-response curve of aortic strips for noradrenaline to the right) did not affect the contractile response to acetylcholine, suggesting that this response is not due to the release of noradrenaline from the sympathetic nerve.

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References

ALHQUIST R.P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.*, **153**, 586–600.

ALTURA, B.M., MALAVIYA, D., REICH, C.F. & ORKIN, L.R. (1972). Effects of vasoactive agents on isolated human umbilical arteries and veins. *Am. J. Physiol.*, **222**, 345–355.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

AXELSSON, J., WOHLSTRÖM, B., JOHANSSON, B. & JONSSON, O. (1967). Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein. *Circulation Res.*, **21**, 609–618.

DE LA LANDE, I.S., CANNELL, V.A. & WATERSON, J.G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. *Br. J. Pharmac., Chemother.*, **28**, 255–272.

DRASKÓCZY, P.R. & TRENDELENBURG, U. (1968). The uptake of *l*- and *d*-norepinephrine by the isolated perfused rabbit heart in relation to the stereospecificity of the sensitizing action of cocaine. *J. Pharmac. exp. Ther.*, **159**, 66–73.

EDMAN, K.A.P. & SCHILD, H.O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. *J. Physiol.*, **161**, 424–441.

FINNEY, D.J. (1964). *Statistical Methods in Biological Assay.* pp. 99–138. London: Charles Griffin and Co.

FLEISCH, A. (1931). Die Wirkung von Histamine, Acetylcholine und Adrenalin auf die Venen. *Pflugers. Arch. Ges. Physiol.*, **228**, 351–372.

FURCHGOTT, R.F. (1967). Pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.*, **139**, 553–570.

FURCHGOTT, R.F. & BHADRAKOM, S. (1953). Relations of strips of rabbit aorta to epinephrine, isopropylalterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

GADDUM, J.H. & HAMEED, K.A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **9**, 240–248.

HUDGINS, P.M. & FLEMING, W.W. (1966). A relatively non-specific supersensitivity in aortic strips resulting from pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **153**, 70–80.

LANGER, S.Z. & TRENDELENBURG, U. (1966). The onset of denervation supersensitivity. *J. Pharmac. exp. Ther.*, **151**, 73–86.

LEWIS, J.H. & KOESSLER, K.K. (1927). Demonstration of arterial contraction *in vitro*. *Archs intern. Med.*, **39**, 182–187.

NAIR, X. & DYER, D.C. (1974). Responses of guinea pig umbilical vasculature to vasoactive drugs. *Eur. J. Pharmac.*, **27**, 294–304.

NUKI, B. (1954). Pharmacology of pulmonary circulation. *Jap. Circul. J.*, **18**, 205–215.

PATON, W.D.M. & VANE, J.R. (1963). An analysis of the response of the isolated stomach to electrical stimulation and to drugs. *J. Physiol.*, **165**, 10–46.

SOMLYO, A.P. & SOMLYO, A.V. (1968). Vascular smooth muscle. *Pharmac. Rev.*, **20**, 197–272.

SOMLYO, A.V., WOO, C.Y. & SOMLYO, A.P. (1965). Responses of nerve-free vessels to vasoactive amines and polypeptides. *Am. J. Physiol.*, **208**, 748–753.

SU, C. & BEVAN, J.A. (1964). Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. *Circulation Res.*, **15**, 20–27.

TAYLER, J. & GREEN, R.D. (1971). Analysis of reserpine-induced supersensitivity in aortic strips of rabbits. *J. Pharmac. exp. Ther.*, **177**, 127–135.

TRENDELENBURG, U. (1962). The action of acetylcholine on the nictitating membrane of the spinal cat. *J. Pharmac. exp. Ther.*, **135**, 39–44.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225–276.

VAN ROSSUM, J.N. (1963). Cumulative dose-response curves. II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Archs int. Pharmacodyn. Ther.*, **143**, 299–330.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VON EULER, U.S. (1938). Action of adrenaline, acetylcholine and other substances on nerve-free vessels (human placenta). *J. Physiol.*, **93**, 129–143.

VON EULER, U.S. (1946). The presence of a sympathomimetic substance in extracts of mammalian heart. *J. Physiol.*, **105**, 38–44.

VON HAGEN, S. & HURWITZ, L. (1967). Effect of extracellular Na and Ca ions on K movement in smooth muscle. *Am. J. Physiol.*, **213**, 579–586.

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