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Interaction between the inhibitory action of acetylcholine and the α-adrenoceptor autoinhibitory feedback system on release of [^{3}H]-noradrenaline from rat atria and rabbit ear artery

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1 Stimulation-induced increases in the efflux of radioactivity (S-I efflux) were measured in the bathing medium following labelling of the noradrenergic transmitter pools of rat atria and rabbit artery preparations with [^{3}H]-noradrenaline.

2 In atria stimulated with trains of 16 or 60 pulses at 2 Hz, phentolamine enhanced, whereas acetylcholine inhibited S-I efflux. With trains of 16 pulses phentolamine had a smaller enhancing effect than with trains of 60 pulses, whereas the inhibitory effect of acetylcholine was more pronounced with 16 pulses of stimulation.

3 The inhibitory effect of acetylcholine was markedly enhanced by phentolamine when stimulation was with 60 pulses. With 16 pulses of stimulation the effect of acetylcholine was unaltered by phentolamine and abolished by the α_2-adrenoceptor agonist 3,4-dihydroxyphenylimino-2-imidazolidine (DPI).

4 Phentolamine had no effect on the negative inotropic effect of acetylcholine on driven left atrial preparations.

5 In arterial preparations stimulated with trains of 30 pulses at 1 Hz, both acetylcholine and clonidine inhibited S-I efflux, whereas yohimbine and idazoxan enhanced S-I efflux. Combining acetylcholine with clonidine did not alter the inhibitory effect of clonidine but the combination of acetylcholine with yohimbine or idazoxan abolished the marked enhancing effects of yohimbine or idazoxan on S-I efflux.

6 These findings indicate that there may be a reciprocal interaction between prejunctional α-adrenoceptors and prejunctional muscarinic cholinoceptors.

Introduction

It has repeatedly been shown that the release of the transmitter noradrenaline from sympathetic nerve terminals may be modulated by endogenous and exogenous substances acting at receptor sites associated with the nerve terminals (Langer, 1977; Starke, 1977; Westfall, 1977; Muscholl, 1979; 1980; Rand *et al.*, 1980). The prejunctional receptors at peripheral noradrenergic neuroeffector sites which have been most thoroughly studied are the inhibitory α-adrenoceptors, the facilitatory β-adrenoceptors and the inhibitory muscarinic cholinoceptors. Most studies concerned with the modulation of transmitter noradrenaline release by agents acting on prejunctional receptors have been designed to investigate the effects of activating or blocking one type of prejunctional receptor, under conditions in which the degree of activation of other types of prejunctional receptors subserving opposing or complementary modulation of noradrenaline release are unknown.

It has been suggested by Langer (1977) that the prejunctional α- and β-adrenoceptors are activated concurrently by transmitter noradrenaline, but reservations have been expressed about this possibility (see Majewski, 1983). Feedback activation of the prejunctional β-adrenoceptors can be observed when adrenaline is released as a co-transmitter with noradrenaline which still exerts its usual autoinhibitory action on prejunctional α-adrenoceptors (Majewski *et al.*, 1980; 1981). However, the effect of activation of prejunctional β-adrenoceptors in enhancing noradrenaline release is inversely related to the degree of activation of the prejunctional α-adrenoceptors (Majewski & Rand, 1981).

There have been a number of reviews dealing with

the influence of a cholinergic parasympathetic innervation on transmitter release from adjacent sympathetic noradrenergic nerve terminals (Kosterlitz & Lees, 1972; Story *et al.*, 1975; Fozard, 1979). In tissues with dual innervation, there is close apposition between cholinergic and noradrenergic varicosities, often without intervening Schwann cell processes. There is evidence that the acetylcholine released from cholinergic nerve terminals can activate prejunctional muscarinic cholinoceptors on the noradrenergic nerve terminals and thereby inhibit release of noradrenaline. This has been demonstrated in preparations of rabbit atria with the vagus and sympathetic nerves intact (Löffelholz & Muscholl, 1970), rabbit lung (Mathé *et al.*, 1977), rabbit jejunum (Manber & Gershon, 1979), and in the dog heart *in vivo* (Levy & Blattberg, 1976); Lavalée *et al.*, 1978). The acetylcholine-mediated inhibition of noradrenaline release is likely to occur concurrently with the autoinhibition of noradrenaline release mediated by a feedback action of noradrenaline on prejunctional α-adrenoceptors.

The aim of the present study was to investigate whether different states of activation of prejunctional α-adrenoceptors can alter the effectiveness of acetylcholine, acting on the prejunctional muscarinic cholinoceptors, in inhibiting release of transmitter noradrenaline from sympathetic nerves. The nerve stimulation-induced release of radioactivity from [^{3}H]-noradrenaline-labelled tissues was used as an index of transmitter noradrenaline release. The studies were carried out in two tissues: rat isolated atria, in which there is both parasympathetic cholinergic and sympathetic noradrenergic innervation, and the rabbit ear artery, in which there is only noradrenergic innervation.

Methods

Rat isolated atria

Albino Wistar rats (220–300 g) of either sex were stunned then decapitated and the hearts rapidly removed. The atria were dissected free and placed in an organ bath containing 4 ml of Krebs-Henseleit solution. The solution was repeatedly changed during a 30 min period. The atria were then incubated with (−)-[7,8-^{3}H]-noradrenaline (2.5 μCi ml^{-1}, 0.19 μM) for 20 min and then again repeatedly washed with Krebs-Henseleit solution for 30 min. At this point the atria were field stimulated with a 30 s train of monophasic square wave pulses of 1 ms duration and supramaximal voltage (about 15 V) delivered at a frequency of 1 Hz through platinum electrodes situated on either side of the atria: this 'priming stimulus' was intended to remove loosely bound tritiated compounds. Repeated washing of the atria was continued for 30 min before sampling commenced. In each experiment the intramural nerves in the atria were field stimulated in two periods with identical trains of either 16 or 60 1 ms pulses at supramaximal voltage and a frequency of 2 Hz. The interval between the periods of stimulation was 30 min. Drugs were introduced 52 min before the first period of stimulation and remained present throughout the experiment, and/or 22 min before the second period of stimulation.

In some experiments, the left atrium was isolated and mounted in an organ bath containing 15 ml of Krebs-Henseleit solution under a resting tension of 2 g. The left atrium was electrically paced with square wave pulses of 5 ms duration and 1 to 3 V amplitude at a frequency of 1 Hz delivered across a punctate electrode about 1 mm above the septal border and a second electrode placed beside the atria. After equilibration for 30 min, during which time the atrium was repeatedly washed with Krebs-Henseleit solution, acetylcholine (0.01 to 30 μM) was added cumulatively to the organ bath. The force of the contraction during exposure to each concentration of acetylcholine was expressed as a percentage of that before acetylcholine. After obtaining the first concentration-response curve, phentolamine (1 μM) was added and remained present for the remainder of the experiment, and a second acetylcholine concentration-response curve was obtained. Controls for the time-dependent changes in sensitivity were run in the absence of phentolamine.

Rabbit ear artery preparation

Rabbits (1.5–4.0 kg) of either sex were killed by cervical dislocation and the proximal 2 cm of the central ear artery of each ear was dissected free and cannulated at each end, mounted vertically and superfused (Allen *et al.*, 1973a) at 4 ml min^{-1} with Krebs-Henseleit solution. After equilibration for 30 min, the arteries were incubated for 60 min with (−)-[7,8^{3}H]-noradrenaline (10 μCi ml^{-1}, 1.3 μM) and then perfused and superfused with Krebs-Henseleit solution for 90 min. In each experiment, the perivascular nerves were field stimulated, in two periods separated by 30 min, with trains of 30 monophasic square wave pulses of 1 ms duration and supramaximal voltage (about 15 V) delivered at a frequency of 1 Hz through bipolar circular platinum electrodes fixed at the proximal end of the artery segment. Drugs were infused into the perfusion-superfusion stream at a rate of 54 μl min^{-1} beginning 22 min before the second period of stimulation, and continuing throughout the remainder of the experiment.

Measurement of efflux of radioactivity

In both artery and atrial preparations, consecutive 1 min samples of the perfusate or bathing solution were taken in each stimulation period, commencing

3 min before the onset of stimulation. The stimulation-induced efflux of radioactivity (S-I efflux) for each period of stimulation was determined by subtracting the mean content of radioactivity of the three samples collected before stimulation from the content of radioactivity in each of the four subsequent samples, and summing the differences. Radioactivity was measured in a Packard 3380 or 460 CD liquid scintillation counter and was expressed as disintegrations per minute (d.p.m.) after correcting for counting efficiency by automatic external standardization. The S-I effluxes of radioactivity were expressed in terms of absolute release (d.p.m.) in the case of atrial preparations whereas in artery preparations the S-I efflux in the second period of stimulation was expressed as a percentage of that in the first period.

Krebs-Henseleit solution

The solution had the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25.1, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-glucose 11.1. Disodium edetate (0.067 mM) and ascorbic acid (0.14 mM) were added to retard oxidation of catecholamines. The solution was aerated with a mixture of 95% O_2 in 5% CO_2 and maintained at 37°C.

Drugs

The following drugs were used: acetylcholine perchlorate (British Drug Houses); N-methyl atropine nitrate (Sigma); clonidine hydrochloride (Boehringer Ingelheim); 3,4-dihydroxyphenylimino-2-imidazolidine (DPI; Boehringer Ingelheim); idazoxan hydrochloride (RX 781094; Reckitt & Coleman); phentolamine hydrochloride (Ciba Geigy); yohimbine hydrochloride (Sigma); (−)-[7,8-^{3}H]-noradrenaline hydrochloride (specific activity 10.5–13.0 Ci mmol^{-1}, radioactive concentration 1.0 mCi ml^{-1}) was obtained from the Radiochemical Centre, Amersham.

Tests of statistical significance

The statistical tests used are indicated in the text. In all cases values of P less than 0.05 were taken to indicate significant differences.

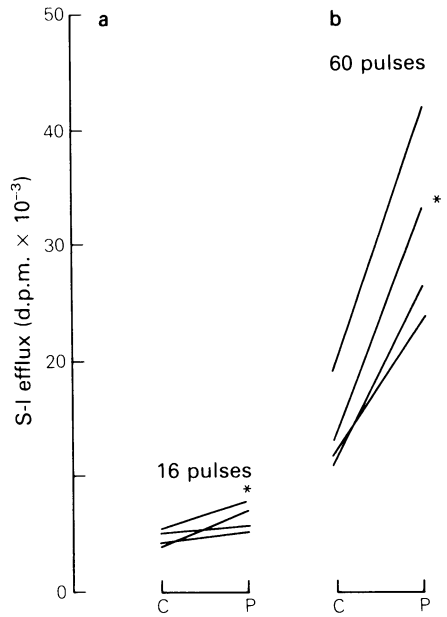

Figure 1 Effect of phentolamine (1 μM) on the increase in S-I efflux of radioactivity from [^{3}H]-noradrenaline-labelled rat atria elicited by field stimulation of intramural sympathetic nerves with (a) 16 and (b) 60 pulses at 2 Hz. The oblique lines join S-I effluxes in the first period of stimulation in the absence (C) of any drug to those in the second period of stimulation, in the presence of phentolamine (P). An asterisk indicates that the mean difference between the S-I effluxes in the first and second periods differs significantly from zero ($P < 0.05$, paired t test, $n = 4$). In control experiments on stimulation with either 16 or 60 pulses the mean difference between the S-I effluxes in the first and second periods was not significant ($P > 0.05$, paired t test).

Results

Rat atria

Control experiments Rat atria were incubated with [^{3}H]-noradrenaline and subjected to two periods of field stimulation with trains of either 16 or 60 pulses at a frequency of 2 Hz. Table 1 shows the resting effluxes

Table 1 Resting and stimulation-induced (S-I) effluxes of radioactivity from [^{3}H]-noradrenaline-labelled rat atria in the first period of stimulation with 16 or 60 pulses

	16 pulses		*60 pulses*	
	Resting efflux (d.p.m. min^{-1})	*S-I efflux* (d.p.m.)	*Resting efflux* (d.p.m. min^{-1})	*S-I efflux* (d.p.m.)
Mean ± s.e.mean	3880 ± 136	4520 ± 247	4272 ± 189	12279 ± 726
n	16	16	22	22

of radioactivity and the stimulation-induced (S-I) effluxes in the first period. The mean S-I efflux of radioactivity induced by stimulation with 16 pulses was 37% of that with 60 pulses. The data in Table 1 are pooled from all experiments in which no drug was present in the first period. In control experiments (no drugs present during either the first or the second period) there was no significant difference between S-I efflux in the first and second periods (paired t test). The S-I efflux for the second period expressed as a percentage of that in the first period had a mean of 115.9% (s.e.mean = 9.9, n = 4) with 16 pulses and 111.1% (s.e.mean = 6.4, n = 4) with 60 pulses.

Effect of phentolamine Phentolamine (1.0 μM) had no effect on the resting efflux of radioactivity but produced a significant enhancement ($P < 0.05$, paired t test) of S-I efflux with either 16 or 60 pulses of stimulation when it was present during the second period (Figure 1). The enhancement of S-I efflux was significantly less ($P < 0.05$, unpaired t test) with 16 pulses than that observed with 60 pulses, indicating that there was less activation of prejunctional α-adrenoceptors with the lower number of pulses.

Effect of acetylcholine Acetylcholine (0.1, 0.3 and 1.0 μM) had no effect on the resting efflux of radioactivity. As shown in Figure 2, in atria stimulated with 60 pulses, acetylcholine, in the highest concentration tested (1.0 μM) significantly reduced S-I efflux when it was added for the second period of stimulation, but in the two lower concentrations (0.1 and 0.3 μM) acetylcholine did not significantly alter S-I efflux (paired t test between S-I effluxes in the first and second periods). When stimulation was with 16 pulses, acetylcholine produced a significant inhibition of S-I efflux at a concentration of 0.3 μM (Figure 3). The inhibitory effect of acetylcholine was observed with a lower concentration when stimulation was with 16 pulses than when it was with 60 pulses, this suggests that inhibition of noradrenaline release by acetylcholine is more pronounced when there is less activation of the prejunctional α-adrenoceptors.

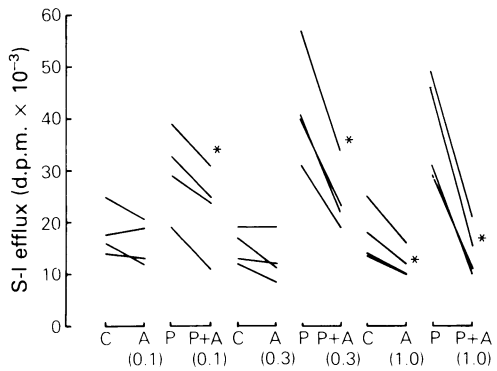

Figure 2 Effect of different concentrations of acetylcholine alone and in the presence of phentolamine (1.0 μM) on the S-I effluxes of radioactivity from [^{3}H]-noradrenaline-labelled rat atria elicited by field stimulation of intramural sympathetic nerves with 60 pulses at 2 Hz. The oblique lines join S-I effluxes in the first period of stimulation in the absence (C) or presence (P) of phentolamine to those in the second period of stimulation in the presence of acetylcholine alone (A) or in the presence of phentolamine and acetylcholine (P + A), respectively. The concentrations of acetylcholine (μM) are given, figure in parentheses. Asterisks indicate significant differences between S-I effluxes in the first and second periods ($P < 0.05$, paired t test, n = 4, in all cases).

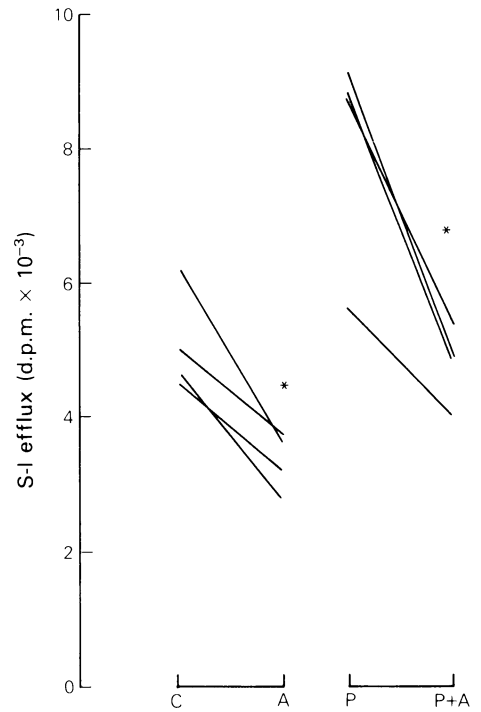

Figure 3 Effect of acetylcholine (0.3 μM) alone and in the presence of phentolamine (1.0 μM) on the S-I effluxes of radioactivity from [^{3}H]-noradrenaline-labelled rat atria elicited by field stimulation of intramural sympathetic nerves with 16 pulses at 2 Hz. The oblique lines join S-I effluxes in the first period of stimulation in the absence (C) of drugs or presence (P) of phentolamine to those in the second period of stimulation in the presence of acetylcholine alone (A) or in the presence of phentolamine and acetylcholine (P + A), respectively. An asterisk indicates that the mean difference between the S-I effluxes in the first and second periods differs significantly from zero ($P < 0.05$, paired t test, n = 4 in all cases).

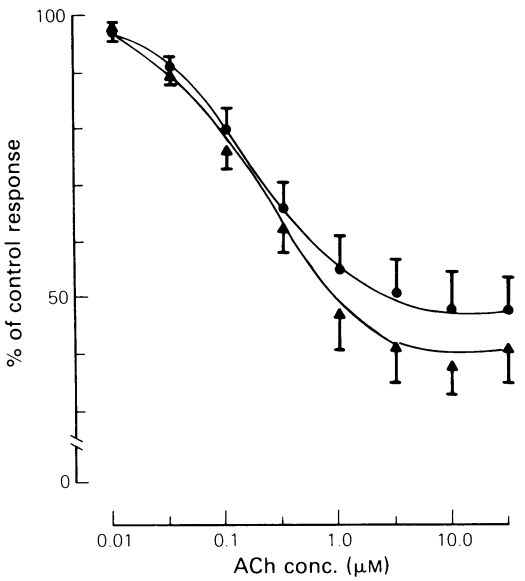

Figure 4 Concentration-response curves for the negative inotropic action of acetylcholine (ACh) on rat left atria driven at a frequency of 1 Hz in the absence (●) and presence (▲) of phentolamine (1 μM). The vertical lines indicate s.e.mean (n = 4).

Effect of acetylcholine in the presence of phentolamine When the prejunctional α-adrenoceptors were blocked with phentolamine (1.0 μM) which was present throughout, the S-I efflux was increased in the first period (Figure 3): with 16 pulses of stimulation, the S-I efflux was about 1.7 fold, and with 60 pulses 2.4 fold, that when no drug was present in the first period. With 60 pulses, the addition of acetylcholine in concentrations of 0.1, 0.3 and 1.0 μM resulted in considerable and significant concentration-dependent inhibition of S-I effluxes in the second period (Figure 2). Thus, the findings suggest that when the autoinhibitory feedback loop mediated by noradrenaline acting on prejunctional α-adrenoceptors was blocked by phenolamine, the inhibitory effect of acetylcholine on noradrenaline release was greatly enhanced. With 16 pulses of stimulation, when the autoinhibitory feedback loop was operating to a lesser extent, the addition of acetylcholine (0.3 μM) in the second period resulted in an inhibitory effect that was not significantly different ($P > 0.05$, unpaired t test) from that produced by acetylcholine in the absence of phentolamine (Figure 3).

Effect of phentolamine on the postjunctional response to acetylcholine The enhancement of the inhibitory effect of acetylcholine on S-I efflux by phentolamine could be due to some other mechanism than blockade of α-adrenoceptors, for example by inhibition of

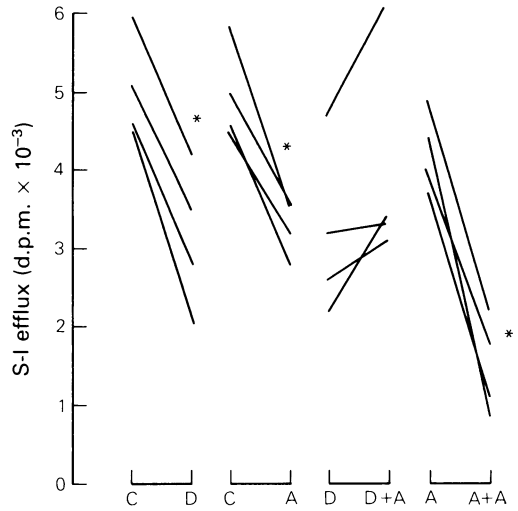

Figure 5 Effects of acetylcholine (0.3 μM) and 3,4-dihydroxyphenylimino-2-imidazolidine (DPI, 0.3 μM), alone and in combination, on the S-I effluxes of radioactivity from [^{3}H]-noradrenaline-labelled rat atria elicited by field stimulation of intramural sympathetic nerves with 16 pulses at 2 Hz. The oblique lines join S-I effluxes in the first period of stimulation in the absence (C) of drugs or in the presence of DPI (D) or acetylcholine (A) to those in the second period of stimulation in the presence of DPI, acetylcholine, DPI and acetylcholine (D + A) or acetylcholine with acetylcholine (A + A). An asterisk indicates that the mean difference between the S-I effluxes in the first and second periods differs significantly from zero ($P < 0.05$, paired t test, n = 4 in all cases).

cholinesterase. Therefore, the effect of phentolamine on the negative inotropic effect of acetylcholine on driven left atrial preparations was investigated. As can be seen in Figure 4, phentolamine (1.0 μM) did not significantly alter the concentration-response curve for this postjunctional action of acetylcholine (0.01–30 μM). The mean log concentrations of acetylcholine producing a half-maximal effect in the absence and presence of phentolamine were 2.48 μM (s.e.mean = 1.58, n = 4) and 3.24 μM (s.e.mean = 0.7, n = 4), respectively.

Effect of 3,4-dihydroxyphenylimino-2-imidazolidine This drug is approximately equipotent with clonidine as an agonist of α_2-adrenoceptors (Medgett, 1984). In some preliminary experiments clonidine was used as an agonist for the atrial prejunctional α_2-adrenoceptors; however, with the longer trains of stimulation, its effects on S-I efflux were inconsistent. This may have been due to clonidine acting as a partial agonist at the atrial prejunctional receptors (Medgett *et al.*, 1978). In atria stimulated with 16 pulses, DPI (0.3 μM) had no effect on resting efflux of radioactivity but produced a

Table 2 Resting and stimulation-induced (S-I) effluxes of radioactivity from [^{3}H]-noradrenaline-labelled rabbit ear arteries in the first period of stimulation at a frequency of 1 Hz for 30 s

	Resting efflux (d.p.m. min^{-1})	*S-I efflux* (d.p.m.)
Mean ± s.e.mean	2164 ± 88	3044 ± 200
n	58	58

significant reduction ($P < 0.05$, paired t test) in S-I efflux when it was present during the second period (Figure 5).

Effect of acetylcholine in the presence of DPI When DPI (0.3 μM) was present throughout, and stimulation was with 16 pulses, the mean S-I efflux in the first period was about 55% of that in the absence of any drug. When acetylcholine (0.3 μM) was added in the second period in the continued presence of DPI, there was no further inhibition of S-I efflux (Figure 5). This finding supports the previous conclusion that activation of prejunctional α-adrenoceptors reduces the inhibitory effect of acetylcholine on noradrenaline release. However, it was possible that the S-I efflux had already been reduced by DPI to such an extent that no further reduction was possible. This was discounted by the finding that when S-I efflux was lowered to the same extent in the first period by the presence of acetylcholine (0.3 μM) throughout, the addition of a further 0.3 μM of acetylcholine in the second period did result in a significant ($P < 0.05$, paired t test), further inhibition of S-I efflux (Figure 5).

Rabbit ear arteries

Control experiments Rabbit ear arteries were incubated with [^{3}H]-noradrenaline and subjected to two 30 s periods of field stimulation at a frequency of 1 Hz. Table 2 shows the resting effluxes and the effluxes of radioactivity induced by field stimulation in the first period. The results were pooled from all experiments. Drugs were introduced after the first period and were present for the second period. In each experiment the S-I efflux of radioactivity in the second period was expressed as a percentage of that in the first. In control experiments (no drugs present in the second period) there was no significant difference between S-I efflux in first and second periods ($P > 0.05$, paired t test). The S-I efflux of radioactivity for the second period expressed as a percentage of that in the first period had a mean value of 106.8% (s.e.mean = 5.0, n = 8).

Effects of clonidine, yohimbine and idazoxan As shown in Figure 6, the presence of clonidine (0.3 μM) during the second period resulted in a significant reduction of S-I efflux in this period, compared to control, whereas yohimbine (1.0 μM) or idazoxan (1.0 μM) when present during the second period produced significant enhancements of S-I efflux ($P <$ 0.05, unpaired t test). These drugs in the above mentioned concentration had no effect on resting efflux.

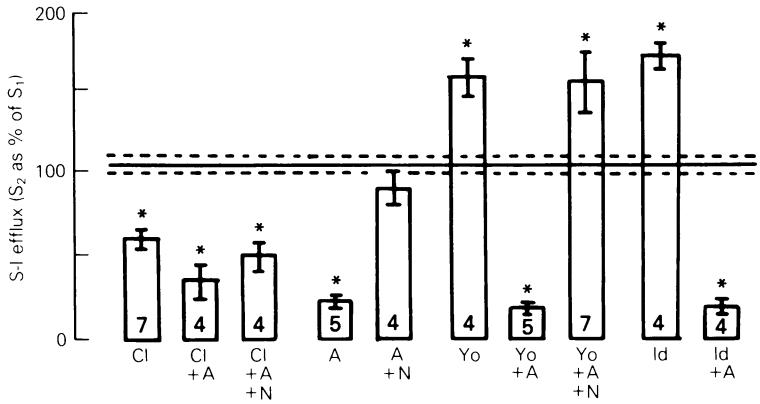

Figure 6 Effects of acetylcholine (A; 0.3 μM), clonidine (Cl; 0.3 μM), yohimbine (Yo; 1.0 μM), idazoxan (Id; 1.0 μM) and N-methyl atropine (N; 0.3 μM) alone or in combination (as indicated below the columns) on S-I efflux, elicited by sympathetic nerve stimulation with 30 pulses at 1 Hz, from [^{3}H]-noradrenaline-labelled rabbit ear arteries. The vertical scale gives the S-I efflux in the second period, during which drugs were present, as a percentage of that in the first period in the absence of drugs (except for N-methyl atropine, when it was used). The heights of columns indicate the mean percentages for the number of experiments given within each column, and s.e.means are indicated by the vertical lines. The horizontal solid and dotted lines indicate the mean and standard error of percentages of 8 experiments in which no drugs were used. Asterisks above columns indicate significant differences from the control mean ($P < 0.05$, unpaired t test).

Effects of acetylcholine Acetylcholine (0.3 μM) introduced in the second period produced a significant reduction in S-I efflux (Figure 5) ($P < 0.05$, unpaired t test) but had no effect on resting efflux of radioactivity. When acetylcholine together with clonidine (0.3 μM) were introduced in the second period, the reduction in S-I efflux was not significantly greater than that produced by clonidine alone. When acetylcholine was introduced together with yohimbine (1.0 μM) or idazoxan (1.0 μM) there were marked reductions in S-I effluxes, despite the enhancement of S-I effluxes produced by yohimbine or idazoxan alone; the reductions of S-I effluxes produced by acetylcholine together with yohimbine or idazoxan were greater than that produced by acetylcholine alone. The effects of acetylcholine in combination with clonidine or yohimbine were abolished by N-methyl atropine (0.3 μM, Figure 6): the S-I effluxes in these experiments did not differ significantly from those in the corresponding experiments in the absence of acetylcholine. Combinations of acetylcholine, yohimbine, idazoxan, clonidine and N-methyl atropine had no significant effect on the resting efflux of radioactivity.

Discussion

The effects of acetylcholine on the efflux of radioactivity elicited by sympathetic nerve stimulation were determined in [^{3}H]-noradrenaline-labelled rat atria and rabbit ear arteries under different states of activation of prejunctional α-adrenoceptors. The stimulation-induced efflux of radioactivity from tissues previously incubated with [^{3}H]-noradrenaline was used as an index of release of transmitter noradrenaline from noradrenergic nerves (Langer, 1970; McCulloch *et al.*, 1974).

The present study provides evidence that the state of activation of prejunctional α-adrenoceptors alters the effectiveness of acetylcholine acting on prejunctional muscarinic cholinoceptors to inhibit transmitter noradrenaline release. The concentrations of acetylcholine used were less than one-hundredth of those required to have any appreciable effect on the uptake of noradrenaline (Allen *et al.*, 1973b), or to have any stimulatory effect on nicotinic cholinoceptors (Muscholl, 1979; 1980). Furthermore, nicotinic cholinoceptors do not appear to modulate the release of [^{3}H]-noradrenaline from rat isolated atria evoked by sympathetic nerve stimulation (Fuder *et al.*, 1982).

In rat atria, the inhibitory effect of acetylcholine on noradrenaline release was examined under two stimulation conditions, namely, with trains of 60 or 16 pulses delivered at 2 Hz. With trains of 60 pulses, there was a greater release of the transmitter than with 16 pulses, and consequently a greater activation of prejunctional α-adrenoceptors, as indicated by the greater effectiveness of phentolamine in enhancing noradrenaline release with 60 than with 16 pulses: in other words, the autoinhibitory feedback effect on noradrenergic transmission was greater with 60 pulses than with 16 pulses of stimulation. When stimulation was with the lower number of pulses, that is, when autoinhibition due to activation of prejunctional α-adrenoceptors was less, the inhibitory effect of acetylcholine on transmitter noradrenaline release occurred with a lower concentration (0.3 rather than 1 μM). Furthermore, on stimulation with 16 pulses the inhibitory effect of acetylcholine was not altered by phentolamine. When stimulation was with 60 pulses, acetylcholine was less potent at inhibiting transmitter noradrenaline release, but the effect of acetylcholine was increased greatly when the prejunctional α-adrenoceptors were blocked by phentolamine. This effect of phentolamine does not appear to be due to enhancement of the action of acetylcholine on muscarinic cholinoceptor sites since phentolamine was without effect on the negative inotropic action of acetylcholine on rat atria. Furthermore, phentolamine does not appear to produce a conformational change in muscarinic cholinoceptor sites, since it has been shown to be without effect on the binding of the cholinoceptor ligand [^{3}H]-quinuclidinyl benzilate to rat atrial membrane preparations (Wei & Sulakhe, 1979). Thus, it appears that activation of prejunctional α-adrenoceptors reduces the inhibitory effect, on transmitter noradrenaline release, of acetylcholine acting on prejunctional muscarinic cholinoceptors. In accord with this suggestion, when there was a relatively low degree of activation of prejunctional α-adrenoceptors by the endogenously released noradrenaline, that is with 16 pulses, the inhibitory effect of acetylcholine was unaffected by phentolamine. Further, when additional activation of prejunctional α-adrenoceptors to that provided by endogenously released noradrenaline was produced by the agonist drug DPI (Medgett, 1983; Van Oene *et al.*, 1983), the ability of acetylcholine to inhibit noradrenaline release was abolished. This effect is not due to maximal activation of inhibitory mechanisms since acetylcholine was still able to inhibit noradrenaline release when the same degree of inhibition to that produced by DPI was produced by acetylcholine.

Similar findings were obtained from experiments with the rabbit ear artery preparation. When additional activation of prejunctional α-adrenoceptors, to that provided by the endogenously released noradrenaline, was produced by clonidine the ability of acetylcholine to inhibit noradrenaline release was reduced. On the other hand, blockade of prejunctional α-adrenoceptors by the selective antagonists yohimbine (Starke *et al.*, 1975) or idazoxan (RX 781094; Doxey *et al.*, 1983) markedly potentiated the ability of acetylcholine to inhibit noradrenaline release, so much

so that yohimbine and idazoxan failed to produce any enhancement of noradrenaline release in the presence of acetylcholine. The inhibitory effects of acetylcholine and combinations of acetylcholine with clonidine or yohimbine were abolished by N-methyl atropine, indicating the involvement of muscarinic cholinoceptors.

These findings suggest that there may be a reciprocal interaction between prejunctional α-adrenoceptors and prejunctional muscarinic cholinoceptors, such that when the α-adrenoceptors are activated, the activation of muscarinic cholinoceptors is less effective at inhibiting noradrenaline release. Conversely, the inhibitory effects of acetylcholine are proportionately increased by decreasing α-adrenoceptor activation. It is of interest that the interaction between the two prejunctional receptor systems was observed in both the atrial and ear artery preparations. The atria are innervated by both cholinergic and noradrenergic nerves and simultaneous activation of the modulatory α-adrenoceptors and muscarinic cholinoceptors on noradrenergic terminals is a distinct possibility (see Introduction). On the other hand it seems unlikely that the prejunctional muscarinic receptors associated with the noradrenergic terminals in the ear artery preparation are normally activated by acetylcholine since in this tissue, as for most blood vessels, there is no obvious source of acetylcholine.

A possible mechanism involved in the interaction between prejunctional α-adrenoceptors and muscarinic cholinoceptors is provided by the evidence that the decreases in noradrenaline release from noradrenergic sympathetic nerves, caused by the activation of either prejunctional α-adrenoceptors or muscarinic cholinoceptors, are finally mediated by diminishing the availability of calcium ions for stimulus-release coupling (Löffelholz & Muscholl, 1969; Starke & Montel, 1974; Dubey *et al.*, 1975; Göthert, 1977). Thus, the mechanisms of inhibition of transmitter noradrenaline release by the prejunctional α-adrenoceptors and the prejunctional muscarinic cholinoceptors may compete for the utilization of this final common pathway. The intracellular location of such a mechanism, the possible second messenger(s) involved and physiological role of the interaction remain to be elucidated.

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