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EFFECTS OF THE HISTAMINE H_2-RECEPTOR BLOCKING DRUGS BURIMAMIDE AND CIMETIDINE ON NORADRENERGIC TRANSMISSION IN THE ISOLATED AORTA OF THE RABBIT AND ATRIA OF THE GUINEA-PIG

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1 In rabbit aortic strips, concentration-response curves to noradrenaline (NA) were shifted to the right in a parallel and concentration-dependent manner by the α-adrenoceptor blocking drug, phentolamine and also by the histamine H_2-receptor blocking drugs, burimamide and cimetidine. Responses to 5-hydroxytryptamine were not affected by these drugs.

2 Burimamide had the properties of a competitive antagonist of noradrenaline, possessing about one-hundredth the potency of phentolamine. Cimetidine was weaker than burimamide and did not fulfil the requirements for competitive antagonism of noradrenaline.

3 In guinea-pig isolated atria, in which noradrenergic transmitter stores were labelled with [^{3}H]-noradrenaline, phentolamine (3 μM), burimamide (30 μM) and cimetidine (30 μM), in decreasing order of effectiveness, each enhanced stimulation-induced efflux of [^{3}H]-noradrenaline, indicating that their blocking effects on prejunctional α-adrenoceptors in this tissue are in the same order of relative potency as on postjunctional α-adrenoceptors in rabbit aortic strips.

4 In the concentrations used (30 μM), neither burimamide nor cimetidine interfered with the neuronal uptake of noradrenaline. Burimamide, and to a much lesser extent, cimetidine, increased the resting efflux of [^{3}H]-noradrenaline from guinea-pig atria.

5 The effect of clonidine, a partial agonist on prejunctional α-adrenoceptors in guinea-pig atria, in increasing stimulation-induced efflux of [^{3}H]-noradrenaline when stimulated with 150 pulses at 5 Hz was blocked by cimetidine (30 μM) and reversed by phentolamine (3 μM) and burimamide (30 μM).

Introduction

In addition to competitive and specific blockade of histamine H_2-receptors, burimamide and metiamide have been shown to exert certain effects on sympathetically innervated tissues. Thus, burimamide, and to a lesser extent, metiamide, are pressor in the pithed rat (Ganellin & Owen, 1977) and induce contraction of the chronically denervated nictitating membrane of the cat (Brimblecombe, Duncan, Owen, & Parsons, 1976); these effects are abolished either by adrenalectomy or by pretreatment with the α-adrenoceptor blocking drug, phentolamine. In kitten isolated atria, burimamide produced concentration-dependent increases in the rate and force of contraction; these effects are abolished by reserpine pretreatment, and are reduced in the presence of either propranolol or cocaine (Hood, Smy & Weetman, 1975). These studies indicate that burimamide and metiamide have a catecholamine-releasing action. In addition, burimamide, but not metiamide, appears to possess α-adrenoceptor blocking activity. Pressor responses to noradrenaline, but not those to angiotensin (Brimblecombe *et al.*, 1976) were blocked by burimamide and a pA_2 value of 4.71 for burimamide against adrenaline was obtained with the rat seminal vesicle preparation. No reports have appeared in the literature to our knowledge to show whether the newer histamine H_2-receptor blocking drug cimetidine (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975a) exerts effects on sympathetically innervated tissues similar to those of burimamide and metiamide.

Effects of histamine H_2-receptor blocking drugs on α-adrenoceptors must be taken into account in the interpretation of the findings that the acute hypotensive effect of the centrally-acting antihypertensive drug, clonidine, is antagonized by the central administration of metiamide and cimetidine (Karppannen,

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Paakkari, Paakkari, Huotari & Orma, 1976; Paakkari, Paakkari & Karppannen, 1976; Finch, Harvey, Hicks & Owen, 1977). It would thus appear that this interaction may involve central histamine H_2-receptors. However, the antihypertensive action of clonidine has hitherto been considered to be due to its agonistic effect on central α-adrenoceptors (Schmitt, Schmitt & Fenard, 1973; Kobinger, 1975); if the histamine H_2-receptor blocking drugs also blocked α-adrenoceptors it would be premature to implicate central histamine H_2-receptors rather than α-adrenoceptors in the action of clonidine.

The object of the present study was to compare the effects of burimamide and cimetidine with those of phentolamine on peripheral α-adrenoceptors in two test preparations: the isolated aorta of the rabbit, in which noradrenaline acts postjunctionally on α-adrenoceptors to elicit contraction, and the isolated atria of the guinea-pig, in which transmitter noradrenaline acts postjunctionally on β-adrenoceptors to increase the rate and force of contractions and in addition acts prejunctionally on α-adrenoceptors to activate an inhibitory feedback mechanism for transmitter release (Rand, McCulloch & Story, 1975). In guinea-pig atria, clonidine has been shown to act as a partial agonist on prejunctional α-adrenoceptors (Medgett, McCulloch & Rand, 1978).

A preliminary account of this work has already been given (Medgett & McCulloch, 1978).

Methods

Rabbit aortic strips

Rabbits of either sex weighing 2 to 4 kg were killed by a blow on the head. The thoracic aorta was rapidly removed and cut spirally into two strips of approximately 3 × 30 mm. The strips were suspended in 25 ml jacketed organ baths, bathed in Krebs-Henseleit solution at 37°C and continuously gassed with 5% CO_2 in O_2. The Krebs-Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose 11.1 and sodium edetate 0.065. The strips were attached, under an initial tension of 4 g, to Harvard force displacement transducers connected to a Rikadenki potentiometric pen recorder. There was an equilibration period of approximately 2 h, during which time the bathing solution was changed every 10 to 15 min. The sensitivity of the strips was assessed by obtaining near maximal responses to noradrenaline or 5-hydroxytryptamine (5-HT). In control experiments, three cumulative concentration-response curves to noradrenaline or 5-HT were obtained at intervals of about 1 h. The maximal contractions in successive curves increased with time. The mean maxima of the second and third noradrenaline curves were, respectively, 1.18 (s.e. mean 0.01, $n = 10$) and 1.24 (s.e. mean 0.08, $n = 5$) times that of the first curves. Those of the second and third 5-HT curves were, respectively, 1.24 (s.e. mean 0.06, $n = 3$) and 1.28 (s.e. mean 0.08, $n = 3$) times that of the first curves. To assess the effects of phentolamine, burimamide or cimetidine, the drug was added to the bathing solution 30 min before eliciting the subsequent concentration-response curve. In order to allow for the increase in sensitivity of the strips with time, responses were expressed as a percentage of the mean maximal response produced by noradrenaline or 5-HT in control experiments.

Guinea-pig atria

Guinea-pigs of either sex weighing 300 to 500 g were killed by cervical dislocation, exsanguinated and the hearts rapidly removed. The atria were dissected free and suspended between two platinum electrodes in a 2.5 ml jacketed organ bath containing Krebs-Henseleit solution, warmed and gassed as for aortic strips. The force of spontaneous contractions of the atria was recorded on a Brush Mark 250 pen recorder using a high-compliance strain gauge transducer. The atria were subjected to an initial tension of about 1 g. The atria were then allowed to equilibrate for 30 min, during which time the Krebs-Henseleit solution was repeatedly changed.

For measuring transmitter release, the atria were equilibrated and then incubated with [^{3}H]-noradrenaline (4 μCi/ml; 0.4 μM) for 20 min. The atria were then repeatedly washed with fresh drug-free solution for a further period of 57 min. The intramural nerves of the atria were field-stimulated electrically with monophasic square wave pulses of 1 ms duration at a field gradient of 12 V/cm which was supramaximal with respect to the positive inotropic response; the stimulating pulses were monitored on an oscilloscope. The atria responded to electrical stimulation with increases in the rate and force of beating. In each experiment, three successive periods of electrical stimulation with either 5 pulses (1 Hz for 5 s) or 150 pulses (5 Hz for 30 s) were given at 22-min intervals. In the 5 pulse experiments, six consecutive 30 s samples of the bathing solution were taken: two resting samples before electrical stimulation and four during and after electrical stimulation. In the 150 pulse experiments, eight consecutive 1 min samples of the bathing solution were taken: three resting samples, and five during and after electrical stimulation. The stimulation-induced efflux of tritium was calculated by subtracting the mean tritium content in the resting samples from the tritium content in the samples collected during and after simulation was applied. Total radioactivity was measured since

Langer (1970) pointed out that for the calculation of the actual output of transmitter, it is important to include the metabolites and not to rely on the determination of [^{3}H]-noradrenaline alone. When the effect of a drug on the resting and stimulation-induced efflux was to be determined, it was added to the bathing solution 15 min before the second and removed 15 min before the third period of electrical stimulation. In experiments to assess the effect of one drug in the presence of another, the latter drug was added 15 min before the first stimulation period and was present for the remainder of the experiment.

Aliquots of 1 ml of the samples which were collected were added to the counting vials containing approximately 0.2 ml of 6 M HCl and 10 ml of a scintillation solution of the following composition: 5.5 g of 2,5-diphenyloxazole (PPO), 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 333 ml of Triton X made up to 1 litre with toluene. The radioactivity was measured in a Packard liquid scintillation spectrometer and was calculated as Becquerels (Bq = disintegration per second) per sample, corrections being made for counting efficiency by the use of an external reference standard.

To measure the neuronal uptake of noradrenaline, isolated atria of the guinea-pig were prepared and equilibrated as described above. The atria were then incubated for 10 min with [^{3}H]-noradrenaline (4 μCi/ml; 0.4 μM). After incubation, the atria were removed from the organ bath, blotted, weighed and homogenized in 1.5 ml of 0.4 M perchloric acid. The homogenate was allowed to stand for approximately 10 min, centrifuged, and the radioactivity determined in 1.0 ml aliquots of the supernatant. The accumulation of tritium label by the atria during incubation with [^{3}H]-noradrenaline was taken as a measure of the uptake of [^{3}H]-noradrenaline by the intramural sympathetic nerves. The tritium content of 1.0 ml aliquots of the incubation medium, after completion of incubation, was also determined by liquid scintillation counting, and the tissue-medium ratio was then calculated as the ratio of the tissue concentration of radioactivity (Bq per g tissue) to the concentration of radioactivity in the incubation medium (Bq per ml), corrections being made for counting efficiency by the use of an external reference standard. The effects of drugs on the uptake of [^{3}H]-noradrenaline were investigated by pre-incubating atria with the required concentration of the drug for a period of 15 min before the addition of [^{3}H]-noradrenaline; the drug remained in contact with the atria during incubation with [^{3}H]-noradrenaline.

Radiochemicals and drugs.

Tritiated *laevo*-noradrenaline ([7-^{3}H]-(−)-noradrenaline) was obtained either from New England Nuclear Corporation or the Radiochemical Centre, Amersham (specific activity: 5.8 Ci/mmol). The following drugs were used: burimamide (Smith, Kline & French); cimetidine (Smith, Kline & French); clonidine hydrochloride (Boehringer Ingelheim); cocaine hydrochloride (McFarlan Smith); serotonin (5-hydroxytryptamine creatinine sulphate; Koch-Light); (−)-noradrenaline hydrochloride (Sigma); phentolamine mesylate (Ciba). Burimamide and cimetidine were initially dissolved in the minimum volume of 0.1 M HCl. The other drugs were initially dissolved in distilled water; in the case of noradrenaline, the distilled water contained sodium edetate (50 μg/ml) and ascorbic acid (50 μg/ml) to retard oxidation. Final dilutions were made in Krebs-Henseleit solution.

Analysis of results and statistics

Regression lines were fitted to the linear portions of concentration-response curves from individual experiments and EC_{50} values (concentrations of agonists required to produce 50% of their own maximum effect) were calculated by the method of least squares; the lines were tested for deviation from linearity. To assess the effect of an antagonist, the type of antagonism and the pA_2 value were determined by the method of Arunlakshana & Schild (1959).

Results were expressed as arithmetic means and the standard error of the mean (s.e. mean), with n as the number of experiments. The unpaired Student's t test was used to test for significant ($P < 0.05$) differences between the means. P values quoted are for two-tail tests. Student's t was calculated from either the combined variance estimate of two samples or from the individual sample variances, depending upon the homogeneity of the sample variances.

Results

Rabbit aortic strips

Mean concentration-response curves for noradrenaline in the absence (control) and presence of three different concentrations of burimamide (1 μM, 10 μM and 100 μM) are shown in Figure 1. Burimamide produced a concentration-dependent displacement to the right of these curves, and analysis of these data shows that, in the presence of any of the three concentrations of burimamide, the curve departed significantly from coincidence but not significantly from parallelism with the control curve.

Cimetidine in the same concentrations as burimamide (1 μM, 10 μM and 100 μM) also produced concentration-dependent displacement to the right of the noradrenaline concentration-response curve (Figure 2). The curves departed significantly from coincidence

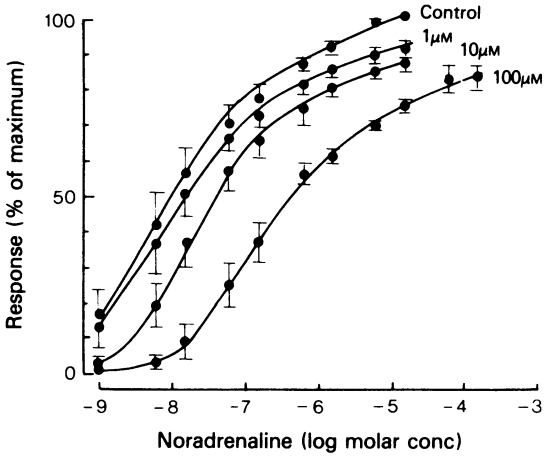

Figure 1 Contractile responses of rabbit aortic strips: mean concentration-response curves to noradrenaline in the absence (control) and in the presence of three different concentrations of burimamide (1 μM, 10 μM and 100 μM). Each point represents the mean of 4 different experiments; the vertical bars represent s.e. mean. The contractile response is expressed as a percentage of the maximum response to noradrenaline, corrected for the increase in the sensitivity of the strips with time.

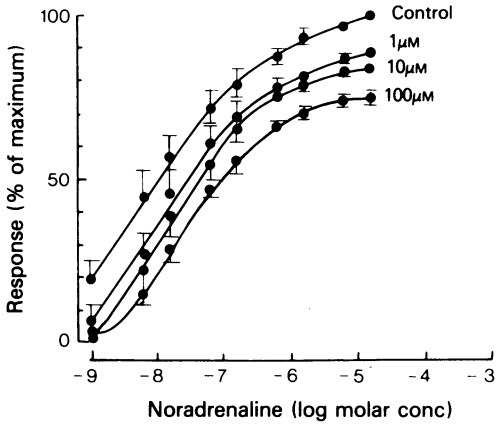

Figure 2 Contractile responses of rabbit aortic strips: mean concentration-response curves to noradrenaline in the absence (control) and presence of three different concentrations of cimetidine (1 μM, 10 μM and 100 μM). Each point represents the mean of 4 different experiments. The vertical bars represent s.e. mean. The contractile response is expressed as a percentage of the maximum response to noradrenaline, corrected for the increase in the sensitivity of the strips with time.

but not from parallelism with the control curve. The concentration-response curve for the contractile effect of 5-HT was not affected by cimetidine in concentrations up to 100 μM.

From Figure 3 it can be seen that burimamide approximates well to a pure competitive antagonist, since the Arunlakshana & Schild plots for both phentolamine and burimamide had slopes significantly greater than zero but not significantly different from one. Thus, as an antagonist of noradrenaline at postjunctional α-adrenoceptors in the rabbit aorta, burimamide possessed a pA_2 value of 5.69 (95% confidence limits = 5.52–5.85), and as such was about one-hundredth as effective as phentolamine, which had a pA_2 value of 7.72 (95% confidence limits = 7.03–8.40). Cimetidine on the other hand did not fulfil the requirements for competitive antagonism; the slope of the Arunlakshana & Schild plot was significantly less than one. The displacement of the noradrenaline concentration-response curve was significantly greater in the presence of burimamide (100 μM) than in the presence of cimetidine (100 μM); thus cimetidine appears to possess weaker blocking actions on α-adrenoceptors in the rabbit aorta than does burimamide.

Guinea-pig atria

The effects of phentolamine, burimamide and cimetidine on stimulation-induced transmitter efflux using stimulation parameters of 150 pulses (5 Hz for 30 s) are shown in Figure 4. Phentolamine (3 μM) increased the stimulation-induced efflux about 5 fold; burimamide (30 μM) was somewhat less effective, producing a 3 fold increase. Cimetidine (1 μM, 3 μM and 30 μM) produced a concentration-dependent increase in stimulation-induced efflux, although even at a concentration of 30 μM the increase of approximately 1.5 fold was much less than that seen with either phentolamine (3 μM) or burimamide (30 μM). The two histograms at the far right of Figure 4 indicate that when the effect of cimetidine (30 μM) was assessed in the presence of phentolamine (3 μM), there was no significant alteration in stimulation-induced transmitter efflux; that is, the previously observed increase was abolished.

The effects of the three drugs on the resting efflux of tritium are shown in Figure 4a. Phentolamine (3 μM) and cimetidine (30 μM) significantly enhanced resting release but the effect was very slight. Burimamide (30 μM) produced a more marked (approximately 1.5 fold) increase in resting release.

The interactions between clonidine and either phentolamine, burimamide or cimetidine on stimulation-induced transmitter efflux are shown in Figure 5. As has been previously reported (Medgett *et al.*, 1978), the effect of clonidine (10 μM) varied with different stimulation parameters; thus, with 5 pulses (1 Hz for 5 s), transmitter efflux was decreased by clonidine whereas it was increased approximately 2 fold with

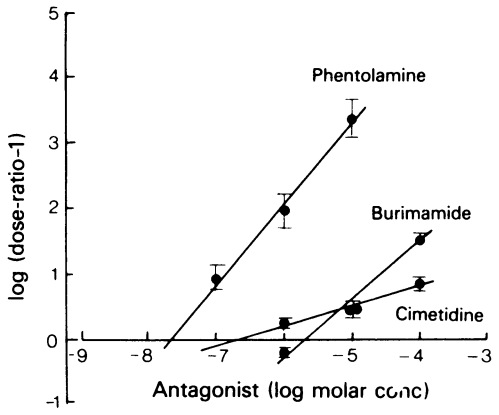

Figure 3 Plots of log (dose-ratio − 1) against log antagonist concentration (Arunlakshana & Schild plot) for the antagonism of responses to noradrenaline by phentolamine, burimamide or cimetidine in the isolated aortic strip of the rabbit. Ordinate scale: log (ratio of mean EC_{50} in presence of antagonist to mean EC_{50} in control experiments minus one). Abscissa scale: log molar concentration of antagonist. Each point represents the mean of either 3 (phentolamine) or 4 (burimamide and cimetidine) different experiments; the vertical bars represent s.e. mean. The linear relationships shown were determined by regression analysis. The regression equations are: phentolamine, $y = 1.19x + 9.22$; burimamide, $y = 0.86x + 4.95$; cimetidine $y = 0.30x + 1.93$. For phentolamine and burimamide, the slopes of the regression lines did not differ significantly from unity, but were significantly greater than zero. For cimetidine, the slope of the regression line was significantly less than unity and significantly greater than zero.

150 pulses (5 Hz for 30 s). However, in the presence of either phentolamine (3 μM) or burimamide (30 μM), and with stimulation parameters of 150 pulses (5 Hz for 30 s), the effect of clonidine (10 μM) was reversed and a decrease in transmitter efflux was observed. In the presence of cimetidine (30 μM), clonidine (10 μM) did not influence transmitter efflux, compared to control.

The effects of phentolamine (3 μM), burimamide (30 μM) and cimetidine (30 μM) were compared with those of the neuronal uptake inhibitor cocaine (30 μM) on the uptake of [^{3}H]-noradrenaline into guinea-pig atria (Figure 6). Cocaine caused an approximately 70% reduction, whereas the other drugs did not influence the uptake of [^{3}H]-noradrenaline.

Discussion

In the isolated aorta of the rabbit, the H_2-receptor blocking drug, burimamide, possesses α-adrenoceptor blocking activity as shown by the displacement of the concentration-response curve to noradrenaline to the right in a parallel and concentration-dependent fashion. The type of antagonism was found to be competitive and a pA_2 value of 5.69 was calculated for burimamide against noradrenaline, which represents a postjunctional α-adrenoceptor blocking activity of approximately one-hundredth that of phentolamine. These results confirm previous reports of the α-adrenoceptor blocking effects of burimamide (Brimblecombe *et al.*, 1976); however, in rabbit aorta the degree of blocking activity appears to be greater than that suggested by the work of Brimblecombe and his colleagues. It was also somewhat surprising to find that the pA_2 value of 5.69 obtained in rabbit aorta for burimamide versus noradrenaline was higher than the pA_2 values of 5.11 and 5.17 reported for burimamide in atria and uterus respectively, by Black, Duncan, Durant, Ganellin & Parsons (1972).

Cimetidine also possesses blocking activity on aortic postjunctional α-adrenoceptors; however, the type of antagonism was not competitive, and the degree of antagonism was less than that of burimamide. Responses to 5-HT were unaffected by cimetidine in concentrations up to 100 μM, which indicates that the effect of cimetidine on contractile responses to noradrenaline is not due to a non-specific relaxant action.

The effects of phentolamine, burimamide and cimetidine on transmitter noradrenaline release from guinea-pig isolated atria suggest that each of the drugs blocks prejunctional α-adrenoceptors which are concerned in an inhibitory feedback mechanism for transmitter release. Although only one concentration of either phentolamine or burimamide was used in these experiments, the observation that a concentration of 3 μM of phentolamine produced a 5 fold enhancement of transmitter efflux in atria compared with a 3 fold enhancement produced by a concentration of 30 μM of burimamide suggests that phentolamine is the more potent antagonist of noradrenaline on atrial prejunctional as well as aortic postjunctional α-adrenoceptors. In the case of cimetidine, there is a concentration-dependent increase in transmitter efflux: 1 μM produced no effect, but 3 μM and 30 μM produced slight but significant increases in efflux. Since a concentration of 1 μM of cimetidine produces a significant degree of α-adrenoceptor blockade in the aorta, it appears that cimetidine acts relatively selectively on these postjunctional α-adrenoceptors rather than the prejunctional α-adrenoceptors of guinea-pig atria. However, on both populations of receptors, cimetidine appears to be a weaker antagonist than burimamide.

The observed order of effectiveness, burimamide being more effective than cimetidine in the above mentioned experiments, argues against the involvement of histamine H_2-receptors rather than α-adreno-

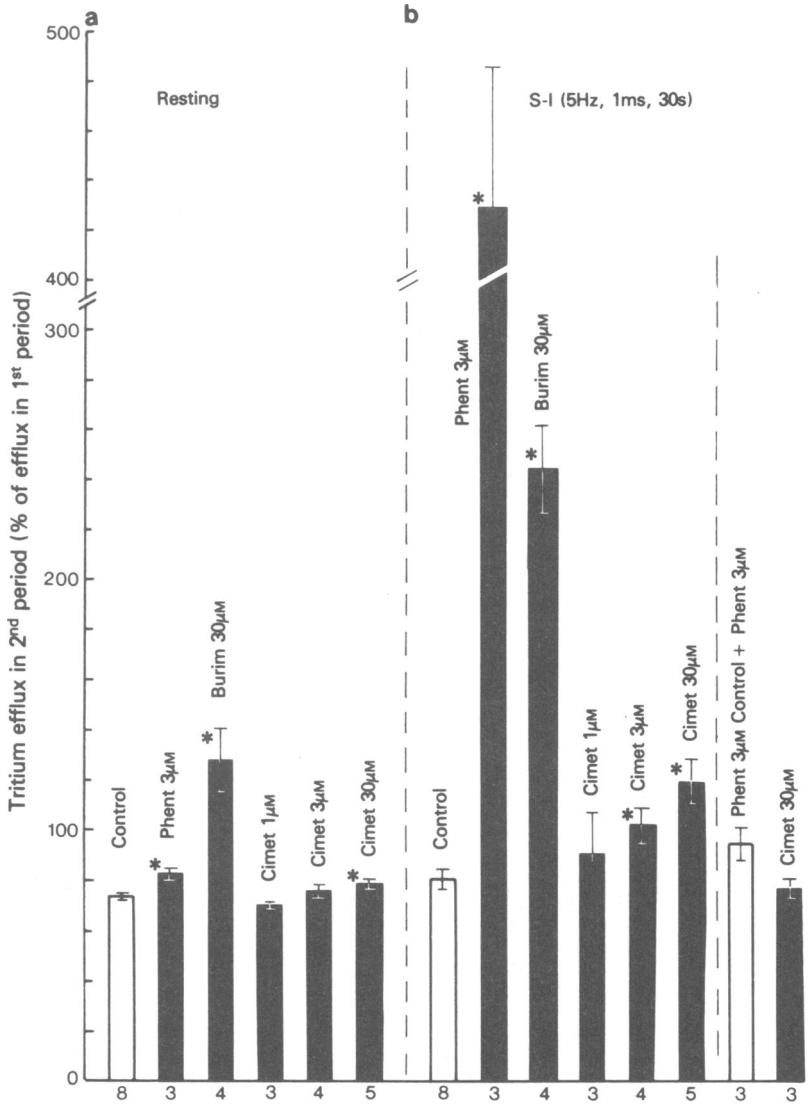

Figure 4 Effects of phentolamine (Phent, 3 μM), burimamide (Burim, 30 μM) and cimetidine (Cimet, 1 μM, 3 μM and 30 μM) on resting (a) and stimulation-induced (S-I) efflux (b) of tritium in guinea-pig isolated atria (solid columns). Stimulation was with 1 ms pulses at 5 Hz for 30 s. Results are expressed as tritium efflux in the second period, during which drugs were present, calculated as a % of the efflux in the first period. The last two histograms describe experiments in which phentolamine (3 μM) was present during all three stimulation periods (Phent 3 μM Control); in some experiments cimetidine (Cimet 30 μM + Phent 3 μM) was also present during the second stimulation period. *Significant difference from the corresponding control experiment (open columns). The number of experiments is given at the base of each column.

ceptors. Cimetidine is reported to be ten times more potent than burimamide as a histamine H_2-receptor antagonist; pA_2 values of 6.1 were obtained for cimetidine on H_2-receptors in both isolated atria and uterus (Brimblecombe, Duncan, Durant, Ganellin, Parsons & Black, 1975b).

Blockade of neuronal uptake is one means whereby stimulation-induced transmitter efflux may be

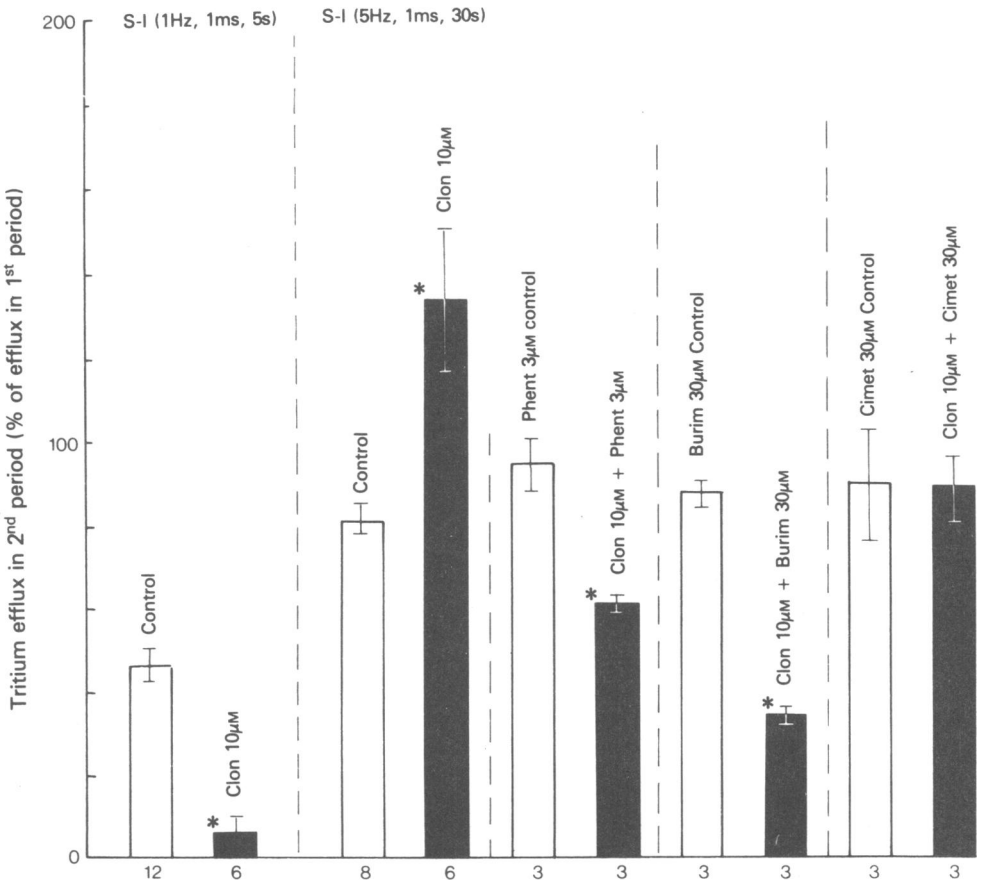

Figure 5 Effect of clonidine (Clon, 10 μM, solid columns) on stimulation-induced efflux of tritium in guinea-pig isolated atria using stimulation parameters of 1 Hz, 1 ms for 5 s and 5 Hz, 1 ms for 30 s. In the experiments using 5 Hz for 30 s stimulation, in some cases the effect of clonidine (10 μM) was assessed in the presence of either phentolamine (Clon 10 μM + Phent 3 μM), burimamide (Clon 10 μM + Burim 30 μM) or cimetidine (Clon 10 μM + Cimet 30 μM). See legend to Figure 4 for further details.

enhanced, as is in fact seen with cocaine in guinea-pig isolated atria (McCulloch, Rand & Story, 1972). However, the neuronal uptake of noradrenaline is not affected by phentolamine, burimamide or cimetidine in the concentrations producing α-adrenoceptor blocking effects, whereas cocaine (30 μM) caused approximately 70% inhibition.

The effect of cimetidine (30 μM) in enhancing stimulation-induced transmitter efflux was abolished when the experiments were repeated in the presence of phentolamine (3 μM); it seems likely that the strong blocking activity of phentolamine on α-adrenoceptors obscures the much weaker effect of cimetidine.

Burimamide (30 μM) produced a significant enhancement of resting release from guinea-pig isolated atria which had previously been incubated with [^{3}H]-noradrenaline. This observation is in accord with previous reports of a catecholamine-releasing action (Albinus & Sewing, 1974; Hood, *et al.*, 1975; Brimblecombe *et al.*, 1976; Ganellin & Owen, 1977). Cimetidine (30 μM) also significantly enhanced the resting efflux of tritium, although the effect was very slight compared with that of burimamide. Ganellin & Owen (1977) presented evidence that the pressor activity of burimamide and three structural analogues was primarily dependent on the basicities of the compounds. Cimetidine, having a pK_a value of 6.8 (Brimblecombe *et al.*, 1975b) is less basic than burimamide which has a pK_a value of 7.8 (Ganellin & Owen, 1977). Hence, it might be expected that cimetidine would be less effective than burimamide in causing catecholamine release; the results obtained in the present study with guinea-pig isolated atria confirm this prediction.

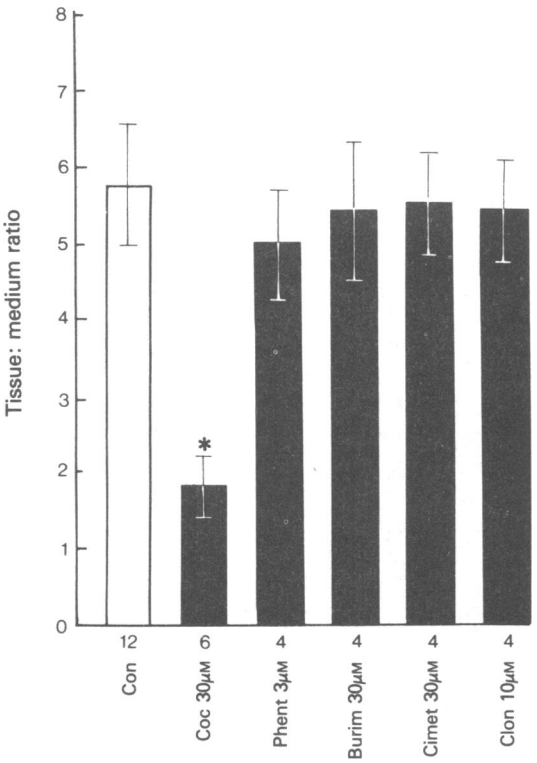

Figure 6 Effects of cocaine (Coc, 30 μM), phentolamine (Phent, 3 μM), burimamide (Burim, 30 μM), cimetidine (Cimet, 30 μM) and clonidine (Clon, 10 μM) on the uptake of [^{3}H]-noradrenaline in guinea-pig isolated atria, expressed in terms of the tissue-medium ratio (see Methods). *Significant difference from control (Con, open column). The number of experiments is given at the base of the columns.

The antihypertensive effects of clonidine have been considered to be due to activation of central α-adrenoceptors (Schmitt *et al.*, 1973). Anden, Grabowska & Strombom (1976), having reviewed the evidence, concluded that the central α-adrenoceptors activated by clonidine resemble prejunctional rather than postjunctional α-adrenoceptors in peripheral tissues. It therefore seemed appropriate to study the interaction between the effects of clonidine and those of either phentolamine, burimamide or cimetidine in guinea-pig atria where the α-adrenoceptors are of the prejunctional type. It has been suggested (Medgett *et al.*, 1978) that when biophase levels of transmitter noradrenaline are maximal (using stimulation parameters of 150 pulses at 5 Hz), the facilitation of transmitter release seen with clonidine (10 μM) in guinea-pig isolated atria is due to a blocking action on prejunctional α-adrenoceptors; such an effect would be expected if clonidine acts as a partial agonist, having a lower intrinsic activity than noradrenaline on prejunctional α-adrenoceptors. In the central nervous system, clonidine has been shown to posses α-adrenoceptor blocking activity; clonidine antagonizes noradrenaline-induced accumulation of cyclic adenosine 3',5'-monophosphate in rat cerebral cortex slices to a degree which corresponds to the 'α-adrenergic component' of the response (Skolnick & Daly, 1975).

Thus in the present study, experiments were carried out to assess the effect of clonidine on transmitter release using stimulation with 150 pulses at 5 Hz in the presence of either phentolamine (3 μM), burimamide (30 μM) or cimetidine (30 μM). Phentolamine and burimamide reversed the effect of clonidine on transmitter release; that is, a decreased release was observed compared with the previously seen facilitation. Cimetidine abolished but did not reverse the effect of clonidine. It is likely that in the presence of an appreciable degree of prejunctional α-adrenoceptor blockade induced by phentolamine or burimamide, occupancy by noradrenaline of the prejunctional α-adrenoceptors is effectively reduced and clonidine, presumably possessing a greater agonist potency and intrinsic activity than either phentolamine or the H_2-receptor blocking drugs at the prejunctional α-adrenoceptors, manifests an α-agonistic action, resulting in a decreased release of noradrenaline. The effect of cimetidine in abolishing rather than reversing the effect of clonidine may reflect its weaker blocking effects on prejunctional α-adrenoceptors. It is possible that a similar interaction between clonidine and histamine H_2-receptor antagonists occurs in the central nervous system. In this case it would seem to be premature to implicate histamine H_2-receptors in the antihypertensive effects of clonidine on the basis of studies with histamine H_2-receptor blocking drugs that have α-adrenoceptor blocking activity.

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