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Interaction of the renin–angiotensin system, bradykinin and sympathetic nerves with cholinergic transmission in the rat isolated trachea

[1,*]Maurice E. Fabiani, *Diem T. Dinh & David F. Story

Pharmacology Research Group, Department of Medical Science, Faculty of Biomedical and Health Sciences, RMIT University Melbourne VIC 3001, Australia

1 The present study was undertaken to investigate the interaction of the renin–angiotensin system (RAS), bradykinin and the sympathetic nervous system with cholinergic transmission in the rat airways. Experiments were performed on epithelium-intact and epithelium-denuded preparations of rat isolated trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores. Tracheal preparations were subjected to electrical field stimulation (trains of 1 ms pulses, 5 Hz, 15 V) and the stimulation-induced (S-I) efflux taken as an index of transmitter acetylcholine release.

2 In both epithelium-intact and epithelium-denuded tracheal preparations, the α_2-adrenoceptor agonist UK14304 (0.1 and 1 μM) inhibited the S-I efflux, in a concentration-dependent manner. The inhibition of S-I efflux produced by UK14304 (1 μM) was antagonized by the selective α_2-adrenoceptor antagonist idazoxan (0.3 μM). Idazoxan (0.3 μM) alone had no effect on the S-I efflux.

3 Angiotensin II (0.1 and 1 μM) was without effect on the S-I efflux in either epithelium-intact or epithelium-denuded tracheal preparations. When angiotensin-converting enzyme was inhibited by perindoprilat (10 μM), angiotensin II (1 μM) was also without effect on the S-I efflux. Similarly, in the presence of idazoxan (0.3 μM), to block prejunctional α_2-adrenoceptors, angiotensin II (0.1 and 1 μM) did not alter the S-I efflux. When added alone, perindoprilat (10 μM) did not alter the S-I efflux.

4 In epithelium-denuded preparations, bradykinin (0.01–1 μM) inhibited the S-I efflux. In epithelium-intact preparations, there was also a tendency for bradykinin (0.1 and 1 μM) to inhibit the S-I efflux but this was not statistically significant. However, when angiotensin-converting enzyme and neutral endopeptidase were inhibited by perindoprilat (10 μM) and phosphoramidon (1 μM), respectively, bradykinin (1 μM) significantly inhibited the S-I efflux in epithelium-intact preparations as well as in epithelium-denuded preparations. The inhibition of the S-I efflux produced by bradykinin, in the combined presence of perindoprilat (10 μM) and phosphoramidon (1 μM), was unaffected by the additional presence of the cyclo-oxygenase inhibitor indomethacin (10 μM) and/or the nitric oxide synthase inhibitor N^G-nitro-L-arginine (100 μM), in either epithelium-intact or epithelium-denuded preparations.

5 In conclusion, the findings of the present study suggest that airway parasympathetic nerves are endowed with α_2-adrenoceptors which subserve inhibition of transmitter acetylcholine release. Under the present conditions, however, transmitter acetylcholine release is not subject to transneuronal modulation by noradrenaline released from adjacent sympathetic nerves in the airways. Moreover, angiotensin II and perindoprilat do not appear to modulate acetylcholine release from parasympathetic nerves of the airways. In contrast, bradykinin inhibits acetylcholine release from airway parasympathetic nerves but this action of bradykinin is limited by the activity of epithelial angiotensin-converting enzyme and/or neutral endopeptidase. The inhibitory action of bradykinin on cholinergic transmission in the airways does not appear to involve the liberation of prostaglandins or nitric oxide.

Keywords: Renin-angiotensin system; angiotensin II; angiotensin-converting enzyme; bradykinin; neutral endopeptidase; prejunctional α_2-adrenoceptors; sympathetic nerves; cholinergic transmission; rat airways

Introduction

The renin–angiotensin system (RAS) plays an integral role in cardiovascular homeostasis by influencing vascular tone, fluid and electrolyte balance, and the activity of the sympathetic nervous system. The biological actions of the RAS are largely mediated by the highly active octapeptide angiotensin II. The RAS has traditionally been viewed as a systemic endocrine system in which the proteolytic enzyme renin, released from the juxtaglomerular cells of the kidney, cleaves angiotensinogen, a liver-derived α_2-globulin, to form the decapeptide angiotensin I which is then subsequently converted to angiotensin II by angiotensin-converting enzyme (ACE), primarily within the pulmonary circulation. However, it should be noted that ACE is not highly specific and is also capable of degrading bradykinin to inactive fragments. In addition to the well-known systemic RAS, there is now substantial evidence to indicate that local formation of angiotensin II also occurs in many effector tissues, including vasculature, heart, kidney and brain (Dzau, 1987, 1988, 1989; Campbell, 1987). Numerous studies have shown that all the requisite components of the RAS such as renin, angiotensinogen and ACE are present in such tissues (Dzau, 1987, 1988, 1989; Campbell, 1987).

The actions of angiotensin II on sympathetic nerves are well documented in the literature. It is well established that angiotensin II can facilitate sympathetic transmission by enhancing the release of noradrenaline from peripheral sympathetic nerve terminals and also by amplifying postjunctional vaso-

[1] Author for correspondence: Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg VIC 3084, Australia.
*Present address: Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg VIC 3084, Australia.

constrictor responses to noradrenaline (Story & Ziogas, 1987; Rand *et al*., 1990). Less is known about the prejunctional actions of angiotensin II on cholinergic nerve terminals. It has been shown that angiotensin II inhibits potassium-evoked release of [^{3}H]-acetylcholine from rat entorhinal cortex (Barnes *et al*., 1989). Similarly, in human temporal cortex, angiotensin II has been reported to inhibit potassium-induced release of [^{3}H]-acetylcholine (Barnes *et al*., 1990; 1992). Further evidence, albeit scant, for possible prejunctional actions of angiotensin II on cholinergic transmission has been provided in functional studies. For instance, it has been shown that angiotensin II inhibits vagal cholinergic neuroeffector transmission by a prejunctional mechanism *in vivo* in dog heart (Potter, 1982a, b) and rabbit stomach (Hobbs & Potter, 1985), and *in vitro* in guinea-pig atria (Potter, 1982a) and dog trachea (Hobbs & Potter, 1985). The peptide has also been shown to inhibit cardiac vagal activity in the pithed rat and guinea pig (Rechtman & Majewski, 1993). In contrast, in the rabbit trachea *in vitro*, it has been reported that angiotensin II, rather than inhibit, potentiates cholinergic transmission (Tamaoki *et al*., 1992; Yamawaki *et al*., 1992).

Although the RAS plays an important role in normal cardiovascular homeostasis, overactivity of the RAS has been implicated in the development of various cardiovascular diseases such as hypertension, congestive heart failure, myocardial infarction and renal disease (Kang *et al*., 1994). Therefore, drugs which interfere with the RAS, such as ACE inhibitors and angiotensin AT_1-receptor antagonists, have been shown to be of therapeutic benefit in the treatment of such cardiovascular disorders (Kang *et al*., 1994; Timmermans & Smith, 1994). Although ACE inhibitors are effective antihypertensive agents, they frequently produce cough which is often their limiting factor with therapy (Semple, 1995). ACE inhibitor-induced cough is thought to be related to increased bronchial reactivity due to the accumulation of bradykinin and other possible proinflammatory mediators in the airways, such as substance P and prostaglandins, as a consequence of their impaired degradation after ACE inhibition (Semple, 1995). Recently, it has been proposed that reduced degradation of these proinflammatory mediators in the airways may lead to stimulation of vagal nerve fibres, thus initiating cough (Semple, 1995).

Bradykinin is a biologically active nonapeptide formed by proteolytic cleavage of the precursor kininogen by the enzyme kallikrein. Bradykinin is involved in a variety of biological processes including the regulation of cardiovascular function, inflammation and pain (Regoli & Barabe, 1980; Lindsey *et al*., 1988). Moreover, there is increasing evidence to suggest that kinins may play a pathological role in various airway disease states including asthma (Barnes *et al*., 1988; Regoli *et al*., 1993). Previous studies have shown that, in the guinea pig trachea, bradykinin causes relaxation of tracheal smooth muscle in the presence of the airway epithelium (Farmer *et al*., 1987; Nijkamp & Folkerts, 1987; Bramley *et al*., 1990; Schlemper & Calixto, 1994, 1995). In contrast, when the epithelium is removed, bradykinin produces contraction of guinea pig airway smooth muscle (Nijkamp & Folkerts, 1987; Farmer *et al*., 1987; Bramley *et al*., 1990). It was postulated that bradykinin-induced relaxation of airway smooth muscle was due to epithelium-derived arachidonic acid products or nitric oxide (Nijkamp & Folkers, 1987; Farmer *et al*., 1987; Schlemper & Calixto, 1994, 1995).

Bradykinin has been shown to enhance noradrenergic transmission in rat (Böke & Malik, 1983) and human (Rump *et al*., 1995) kidney, rat and mouse vas deferens (Llona *et al*., 1991), pithed rat (Dominiak *et al*., 1992), rat hypothalamus (Tsuda *et al*., 1993) and rat atria (Chulak *et al*., 1995). In contrast, bradykinin has been reported to inhibit noradrenergic transmission in rabbit (Malik & Nasjletti, 1979) and canine kidney (Susic *et al*., 1981) and in rabbit pulmonary artery and heart (Starke *et al*., 1977), possibly by a mechanism involving the liberation of inhibitory prostaglandins. Very little information exists pertaining to the actions of bradykinin on cholinergic nerves. In one study, bradykinin was shown to facilitate the release of [^{3}H]-acetylcholine from guinea pig myenteric plexus (Yau *et al*., 1986).

Parasympathetic nerves plays an important functional role in modulating airway smooth muscle tone (Barnes, 1986, 1992). Acetylcholine promotes bronchoconstriction by activating muscarinic cholinoceptors on bronchial smooth muscle. Although well endowed with β-adrenoceptors, there is no evidence of direct sympathetic innervation of airway smooth muscle (Barnes, 1986, 1992). However, sympathetic nerves may play an important neuromodulatory role in regulating the release of acetylcholine from parasympathetic nerves (Barnes, 1986, 1992). Moreover, the epithelium plays an important role in modulating the responsiveness of airway smooth muscle to various bronchoactive agents (Farmer, 1987; Vanhoutte, 1987; Sparrow *et al*., 1995). Recently, it has been shown that the airway epithelium may exert an inhibitory influence on the release of acetylcholine possibly by a mechanism which involves a putative inhibitory factor released from the epithelium (Wessler *et al*., 1990, 1991).

Thus, in view of limited information, the aim of the present study was to investigate the interaction of (1) renin–angiotensin system and bradykinin; and (2) sympathetic nerves with cholinergic transmission in the airways, with particular emphasis on the possible modulatory role of the epithelium. Experiments were performed on epithelium-intact and epithelium-denuded preparations of rat isolated trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores.

Methods

Rat isolated tracheal preparations

Sprague-Dawley rats (200–350 g) of either sex were killed by a blow to the head. The chest was opened longitudinally and the trachea from the larynx to the carina, with the oesophagus attached, was removed and transferred to a Petri dish containing physiological salt solution (PSS), maintained at 37°C and continuously gassed with a mixture of 95% CO_2 and 5% O_2. The oesophagus was carefully separated and a section of the trachea, approximately 2 cm in length, was dissected free. The isolated segment of trachea was either left intact or opened longitudinally by cutting the cartilaginous rings directly opposite the bronchial smooth muscle strip.

In opened tracheal preparations, the epithelium was removed by gently rubbing the luminal surface with a cotton-tipped applicator. The effectiveness of this technique for removal of the epithelium has previously been confirmed by light microscopy of histological transverse sections of tracheal preparations stained with haematoxylin and eosin (Fabiani *et al*., 1996). Both ends of opened and unopened tracheal preparations were tied with silk threads. Each tracheal preparation was initially placed between two platinum wire electrodes in a glass-jacketed organ bath containing 2 ml PSS, maintained at 37°C and continuously gassed with a mixture of 95% CO_2 and 5% O_2.

Radiolabelling of cholinergic transmitter stores

The cholinergic transmitter stores of rat tracheal preparations were radiolabelled according to the procedure described by Fabiani *et al*. (1996). Briefly, the tracheal preparations were equilibrated for 30 min in 2 ml PSS containing non-radioactive choline chloride (1 μM), in a glass-jacketed organ bath. During the last 10 min of this equilibration period, the tracheal preparations were subjected to continuous electrical field stimulation (1 ms monophasic square wave pulses, 10 Hz, 15 V), delivered by a Grass S88 stimulator. This period of stimulation was intended to 'turn over' the acetylcholine transmitter pools to allow more effective incorporation of [^{3}H]-choline into [^{3}H]-acetylcholine (Lindmar *et al*., 1980; Muscholl & Muth, 1982;

Wetzel & Brown, 1983; Loiacono & Story, 1986; Fabiani *et al*., 1996). Immediately after this equilibration period, the tracheal preparations were incubated with [^{3}H]-choline (8.1 μCi/mL; total concentration of 1.1 μM) for 30 min in order to label their cholinergic transmitter stores. During incubation, the tracheal preparations were stimulated continuously (1 ms pulses, 10 Hz, 15 V).

After the labelling procedure, the tracheal preparations were removed from the organ bath and mounted vertically between two platinum wire electrodes in an acrylic flow chamber and continuously superfused with PSS at a rate of 2 ml min^{-1} using a peristaltic pump (Gilson Minipuls 3). To remove loosely-bound radioactivity, each preparation was superfused for 60 min before experimental procedures were commenced. The PSS superfusing the tracheal preparations contained hemicholinium-3 (10 μM), non-radioactive choline chloride (1 μM) and atropine (1 μM). The non-radioactive choline was intended to assist in clearing [^{3}H]-choline from the tissues during the course of the experiment (Dieterich *et al*., 1978; Loiacono & Story, 1986; Fabiani & Story, 1995; Fabiani *et al*., 1996). Hemicholinium-3 was present to prevent the re-uptake of [^{3}H]-choline, originating from hydrolysis of released [^{3}H]-acetylcholine, and also to prevent the uptake of non-radiolabelled choline. Moreover, the muscarinic antagonist atropine was present to block autoinhibition and thus augment the neuronal release of radiolabelled acetylcholine (Fabiani *et al*., 1996). After the first 30 min of the 'washout' period, a 'priming stimulus' was applied for 30 s (1 ms pulses, 1 Hz, 15 V) to remove non-specifically bound radioactive materials.

Stimulation of intramural parasympathetic nerves

After the 'washout' period, the intramural cholinergic nerves of the tracheal preparations were subjected to two 30 s periods of electrical field stimulation (1 ms pulses, 5 Hz, 15 V), delivered 30 min apart. The effects of drugs on the resting and stimulation-induced (S-I) effluxes of radioactivity were investigated by adding the drugs to the PPS superfusing the tracheal preparations 15 min prior to the second period of stimulation. The drugs then remained present for the rest of the experiment. In other experiments, the α_2-adrenoceptor antagonist idazoxan (0.3 μM), the ACE inhibitor perindoprilat (10 μM), the neutral endopeptidase inhibitor phosphoramidon (1 μM), the nitric oxide synthase inhibitor N^G-nitro-L-arginine (100 μM), and/or the cyclo-oxygenase inhibitor indomethacin (10 μM) were added to the PSS either 30 min before the first period of stimulation, or 15 min before the second period of stimulation, and then remained present for the duration of the experiment.

Determination of resting and S-I effluxes of radioactivity

The superfusate from the tracheal preparations was collected at 2 min intervals (rate: 2 ml min^{-1}) by an automated fraction collector (ISCO Retriever IV). Each 2 min (4 ml) fraction of superfusate was mixed with 6 ml of Ultima Gold and the radioactivity present was determined by liquid scintillation counting. Corrections for counting efficiency were made by external automatic standardization and the results expressed as disintegrations per minute (d.p.m.).

The resting efflux of radioactivity from the tracheal preparations for each of the two periods of stimulation (R_1 and R_2) was determined from the amount of radioactivity present in the fraction of superfusate collected immediately before stimulation. The S-I efflux of radioactivity for each of the two periods of stimulation (S_1 and S_2) was calculated by subtracting the resting efflux from the content of radioactivity present in each of the three fractions of superfusate collected from the onset of stimulation, and summing the differences. In each experiment, the resting and S-I effluxes for the second period of stimulation were expressed as percentages of the corresponding values for the first period of stimulation (% R_2/R_1 and % S_2/S_1, respectively).

Composition of physiological salt solution

The PSS had the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.45; $NaHCO_3$, 25; KH_2PO_4, 1.03; D-(+)-glucose, 11.1; disodium edetate, 0.067; and ascorbic acid, 0.14.

Drugs and radiochemicals

The following drugs were used: angiotensin II (AUSPEP, Australia); atropine sulphate (Sigma, U.S.A.); bradykinin (AUSPEP, Australia); choline chloride (Sigma, U.S.A.); hemicholinium-3 (Sigma, U.S.A.); idazoxan hydrochloride (Reckitt & Colman, U.K.); indomethacin (Merck, Sharpe & Dohme, Australia); N^G-nitro-L-arginine (Sigma, U.S.A.); perindoprilat (Technologie Servier, France); phosphoramidon (Sigma, U.S.A.); and UK14304 (Pfizer, U.K.).

Stock solutions and intermediate dilutions of all drugs were made in distilled water. Final concentrations of drugs were achieved by the appropriate dilution in PSS, continuously gassed with 95% CO_2 and 5% O_2 and maintained at a temperature of 37°C.

[Methyl-^{3}H]-choline chloride (specific activity 81 Ci/mmol) was supplied by the Radiochemical Centre, Amersham, U.K.

Statistical analysis of results

Results are expressed as mean ± standard error of mean (s.e.mean); n represents the number of experiments. The levels of statistical significance of differences were determined by one-way or two-way analyses of variance (ANOVA) followed by Dunnett's test or Student–Newman–Keuls (SNK) test, where appropriate. All statistical analyses were performed using the statistical program SIGMASTAT® FOR WINDOWS (Jandel Scientific). In all cases, probability levels less than 0.05 ($P<0.05$) were taken to indicate statistical significance.

Results

Resting and S-I effluxes from epithelium-intact tracheal preparations

Control experiments In control experiments with epithelium-intact preparations of rat trachea previously incubated with [^{3}H]-choline, the mean absolute resting efflux of radioactivity for the first period of stimulation (R_1) was 3171 ± 130 d.p.m. per 2 min period ($n=40$). There was a progressive decline in the resting efflux between the first and second period of stimulation. The mean value of the resting efflux of radioactivity for the second period of stimulation, expressed as a percentage of that for the first (% R_2/R_1), was 83.8 ± 2.3% ($n=7$). The tracheal preparations were subjected to two 30 s periods of electrical field stimulation (1 ms pulses, 5 Hz, 15 V), delivered 30 min apart. The mean absolute efflux of radioactivity evoked by the first period of stimulation (S_1) was 12225 ± 851 d.p.m. ($n=40$). The mean value of the S-I efflux of radioactivity for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was 75.0 ± 4.4% ($n=7$).

Effects of UK14304 and idazoxan When introduced 15 min before the second period of stimulation, the selective α_2-adrenoceptor agonist UK14304 (0.1 μM) did not significantly alter the resting efflux (Table 1). There was a tendency for UK14304 (0.1 μM) to reduce the S-I efflux but this was not statistically significant (Figure 1a). However, a higher concentration of UK14304 (1 μM) significantly reduced the S-I efflux (Figure 1a) and slightly but significantly enhanced the resting efflux (Table 1).

The selective α_2-adrenoceptor antagonist idazoxan was used to determine whether the inhibitory effects of UK14304 on S-I efflux were due to activation of α_2-adrenoceptors.

Idazoxan (0.3 μM), when introduced 30 min before the first period of stimulation, did not alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of idazoxan (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered when idazoxan was present (Table 1). Similarly, the absolute value of the S-I efflux with the first period of stimulation (S_1) and the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), were not significantly different when idazoxan was present (Table 2 and Figure 1a, respectively).

Idazoxan (0.3 μM), introduced 30 min before the first period of stimulation, prevented the inhibition of the S-I efflux produced by UK14304 (1 μM) (Figure 1a). In the presence of idazoxan, UK14304 did not alter the resting efflux (Table 1). When introduced alone 15 min before the second period of stimulation, idazoxan (0.3 μM) did not alter either the resting efflux (Table 1) or the S-I efflux (Figure 1a).

Table 1 Effects of drugs on the resting efflux of radioactivity from epithelium-intact preparations of rat isolated trachea previously incubated with [^{3}H]-choline

*Drug**	*Resting efflux (% R_2/R_1)***	*n*
None		
Control	83.8±2.3	7
UK14304 (0.1 μM)	85.3±1.4	4
UK14304 (1 μM)	94.4±2.2†	4
Idazoxan (0.3 μM)	80.5±2.9	5
Angiotensin II (0.1 μM)	94.7±1.4†	4
Angiotensin II (1 μM)	92.9±2.2†	4
Perindoprilat (10 μM)	87.3±1.7	4
Bradykinin (0.1 μM)	86.4±1.4	4
Bradykinin (1 μM)	104.6±3.7†	4
Idazoxan (0.3 μM) present throughout		
Control	83.6±2.3	4
UK14304 (1 μM)	87.6±6.2	4
Angiotensin II (0.1 μM)	87.3±2.6	4
Angiotensin II (1 μM)	81.7±1.3	4
Perindoprilat (10 μM) present throughout		
Control	80.2±3.1	4
Angiotensin II (1 μM)	97.0±4.7†	4
Perindoprilat (10 μM)+Phosphoramidon (1 μM) present throughout		
Control	79.8±3.1	4
Bradykinin (1 μM)	99.4±4.2†	4
Perindoprilat (10 μM)+Phosphoramidon (1 μM)+indomethacin (10 μM) present throughout		
Control	78.7±0.5	4
Bradykinin (1 μM)	109.5±2.3†	5
Perindoprilat (10 μM)+Phosphoramidon (1 μM)+indomethacin (10 μM)+N^G-nitro-L-arginine (100 μM) present throughout		
Control	85.2±3.1	4
Bradykinin (1 μM)	94.8±5.3	4

*See text for details of drug exposure. **Resting efflux for the second period of stimulation was expressed as a percentage of that for the first period of stimulation. Values are mean±s.e.mean. †Significantly different from corresponding control value ($P<0.05$, ANOVA, Dunnett's test)

Effects of angiotensin II and perindoprilat Angiotensin II (0.1 and 1 μM), introduced 15 min before the second period of stimulation, slightly enhanced the resting efflux (Table 1) but did not alter the S-I efflux (Figure 2a).

The ACE inhibitor perindoprilat was used to exclude the possible involvement of locally-formed endogenous angiotensin II. Perindoprilat (10 μM), when introduced 30 min before the first period of stimulation, did not significantly alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of perindoprilat (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered when perindoprilat was present (Table 1). The absolute value of the S-I efflux for the first period of stimulation (S_1) was significantly greater when perindoprilat was present compared with that in its absence (Table 2). However, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly different when perindoprilat was present throughout (Figure 2a).

In the presence of perindoprilat (10 μM), angiotensin II (1 μM) slightly enhanced the resting efflux (Table 1) but did not alter the S-I efflux (Figure 2a). When introduced alone 15 min before the second period of stimulation, perindoprilat (10 μM) did not alter either the resting efflux (Table 1) or the S-I efflux (Figure 2a).

Effects of angiotensin II after blockade of prejunctional α_2-adrenoceptors The selective α_2-adrenoceptor antagonist idazoxan was used to circumvent possible α_2-adrenoceptor-mediated transneuronal modulation of acetylcholine release by noradrenaline released from adjacent sympathetic nerves. In

Table 2 Absolute resting and S-I effluxes of radioactivity from epithelium-intact and epithelium-denuded preparations of rat isolated trachea previously incubated with [^{3}H]-choline

	Epithelium-intact		*Epithelium-denuded*	
*Drug present throughout**	*R_1*	*S_1*	*R_1*	*S_1*
None	3171±130 (*n*=40)	12225±851 (*n*=40)	2778±94 (*n*=42)	3840±318 (*n*=42)
Idazoxan (0.3 μM)	3492±88 (*n*=4)	15064±2682 (*n*=4)	2889±298 (*n*=5)	4334±507 (*n*=5)
Perindoprilat (10 μM)	3743±126 (*n*=4)	20621±1183† (*n*=4)	3613±68† (*n*=4)	6562±854† (*n*=4)
Perindoprilat (10 μM)+phosphoramidon (1 μM)	4368±290† (*n*=4)	17433±3821 (*n*=4)	3596±243† (*n*=4)	5768±1247 (*n*=4)
Perindoprilat (10 μM)+phosphoramidon (1 μM)+indomethacin (10μM)	4223±346 (*n*=4)	17502±5168 (*n*=4)	3978±331† (*n*=4)	10151±984† (*n*=4)
Perindoprilat (10 μM)+phosphoramidon (1 μM)+indomethacin (10 μM)+N^G-nitro-L-arginine (100 μM)	3573±161 (*n*=4)	8025±1655 (*n*=4)	3101±135 (*n*=4)	7250±429† (*n*=4)

*See text for details of drug exposure. Values are mean±s.e.mean. †Significantly different from corresponding value obtained in the absence of drugs ($P<0.05$, ANOVA, Dunnett's test).

the presence of idazoxan (0.3 μM), angiotensin II (0.1 and 1 μM) did not significantly affect the resting (Table 1) or the S-I (Figure 2a) effluxes of radioactivity.

Effects of bradykinin before and after inhibition of ACE and NEP In epithelium-intact tracheal preparations, bradykinin (0.1 and 1 μM), when introduced 15 min before the second period of stimulation, did not significantly alter the S-I efflux (Figure 3a). However, the higher concentration of bradykinin (1 μM) enhanced the resting efflux (Table 1).

As bradykinin is a substrate for both ACE and neutral endopeptidase (NEP), perindoprilat and phosphoramidon were used, respectively, to prevent the degradation of exogenously applied bradykinin. When introduced 30 min before the first period of stimulation, the combination of perindoprilat (10 μM) and phosphoramidon (1 μM), slightly enhanced the absolute resting efflux for the first period of stimulation (R_1), compared with that in their absence (Table 2). However, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the combined presence of perindoprilat and phosphoramidon (Table 1). The absolute value of the S-I efflux for the first period of stimulation (S_1) was not significantly altered when perindoprilat and phosphoramidon were present together (Table 2). Moreover, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly different when perindoprilat and phosphoramidon were present in combination (Figure 3a).

In the combined presence of perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin in a concentration of 1 μM, significantly reduced the S-I efflux (Figure 3a) and enhanced the resting efflux (Table 1).

Effects of bradykinin in the presence of indomethacin The cyclo-oxygenase inhibitor indomethacin was used to determine

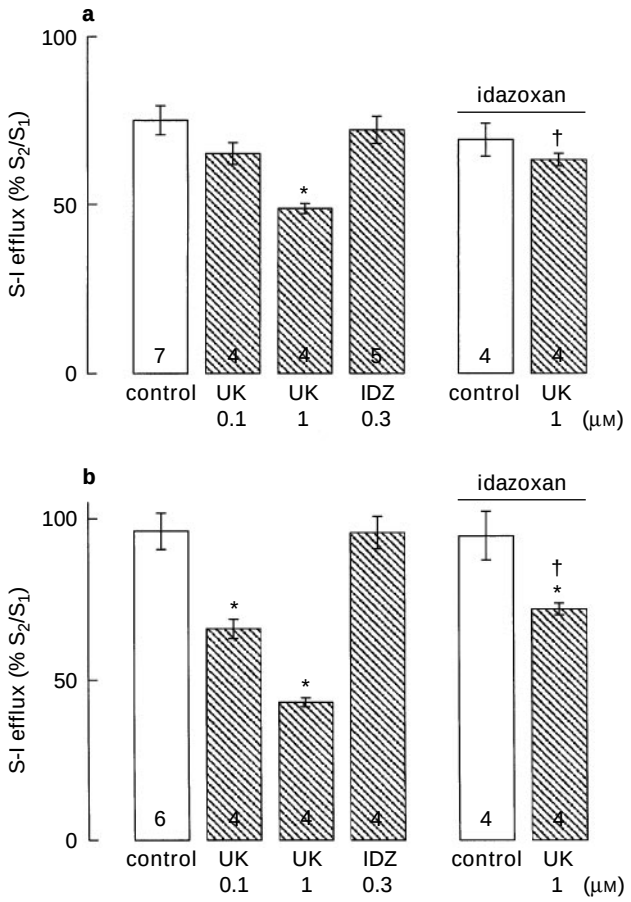

Figure 1 Effects of UK14304, idazoxan, and UK14304 in the presence of idazoxan, on the S-I efflux of radioactivity from epithelium-intact (a) and epithelium-denuded (b) preparations of rat isolated trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores. The intramural parasympathetic nerves of the tracheal preparations were subjected to two periods of electrical field stimulation (5 Hz, 30 s), given 30 min apart. UK14304 (UK, 0.1 or 1 μM) or idazoxan (IDZ, 0.3 μM) were added to the PSS superfusing the tracheal preparations 15 min before the second period of stimulation. In some experiments, idazoxan (0.3 μM) was added to the PSS 30 min before the first period of stimulation and remained present throughout. The S-I efflux for the second period of stimulation was expressed as a percentage of the corresponding value for the first period (% S_2/S_1). Each column represents the mean$\pm$s.e.mean; the number of preparations (n) is indicated at the base of each column. Significant differences from corresponding controls are indicated by asterisks (*$P<0.05$, ANOVA, Dunnett's test). A significant effect of idazoxan is indicated by an obelus (†$P<0.05$, ANOVA, SNK test).

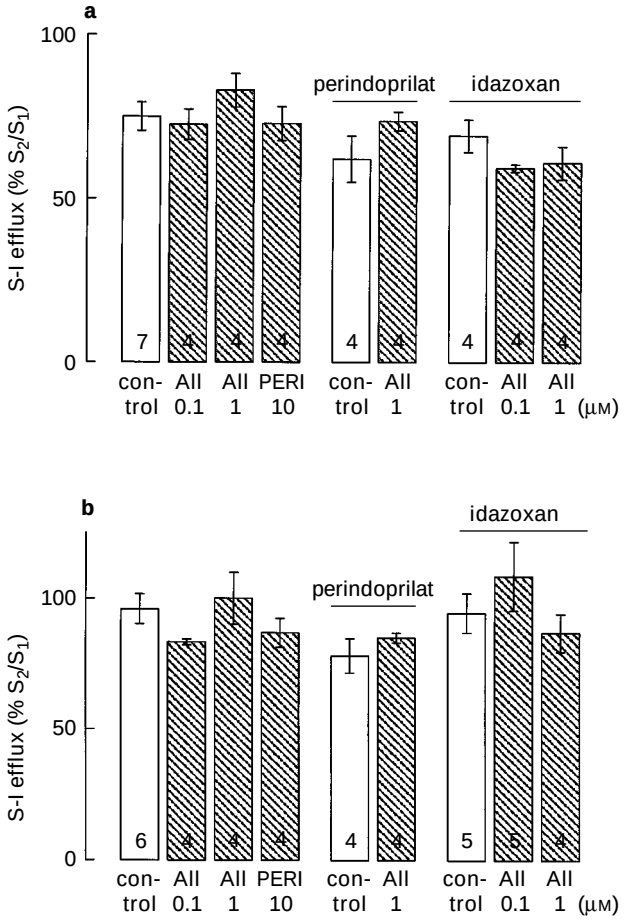

Figure 2 Effects of angiotensin II, perindoprilat, and angiotensin II in the presence of either perindoprilat or idazoxan, on the S-I efflux of radioactivity from epithelium-intact (a) and epithelium-denuded (b) preparations of rat isolated trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores. The intramural parasympathetic nerves of the tracheal preparations were subjected to two periods of electrical field stimulation (5 Hz, 30 s), given 30 min apart. Angiotensin II (AII, 0.1 or 1 μM) or perindoprilat (PERI, 10 μM) were added to the PSS superfusing the tracheal preparations 15 min before the second period of stimulation. In some experiments, perindoprilat (10 μM) or idazoxan (0.3 μM) were added to the PSS 30 min before the first period of stimulation and remained present throughout. The S-I efflux for the second period of stimulation was expressed as a percentage of the corresponding value for the first period (% S_2/S_1). Each column represents the mean$\pm$s.e.mean; the number of preparations (n) is indicated at the base of each column.

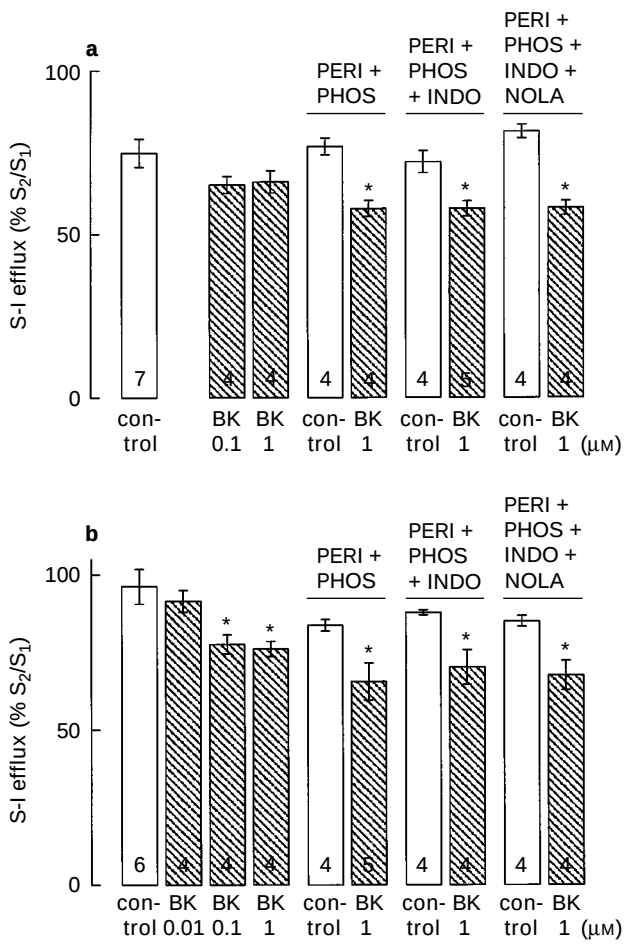

Figure 3 Effects of bradykinin, in the absence and presence of the combinations of perindoprilat and phosphoramidon, or perindoprilat, phosphoramidon and indomethacin, or perindoprilat phosphoramidon, indomethacin and N^G-nitro-L-arginine, on the S-I efflux of radioactivity from epithelium-intact (a) and epithelium-denuded (b) preparations of rat isolated trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores. The intramural parasympathetic nerves of the tracheal preparations were subjected to two periods of electrical field stimulation (5 Hz, 30 s), given 30 min apart. Bradykinin (BK, 0.01–1 μM) was added to the PSS superfusing the tracheal preparations 15 min before the second period of stimulation. Perindoprilat (PERI, 10 μM), phosphoramidon (PHOS, 1 μM), indomethacin (INDO, 10 μM) and/or N^G-nitro-L-arginine (NOLA, 100 μM) were added to the PSS 30 min before the first period of stimulation and remained present throughout. The S-I efflux for the second period of stimulation was expressed as a percentage of the corresponding value for the first period (% S_2/S_1). Each column represents the mean $\pm$ s.e.mean; the number of preparations (n) is indicated at the base of each column. Significant differences from corresponding controls are indicated by asterisks (*$P<0.05$, ANOVA, Dunnett's test).

whether the inhibitory effect of bradykinin on S-I efflux was due to the formation of prostaglandins. Indomethacin (10 μM), when introduced together with perindoprilat (10 μM) and phosphoramidon (1 μM), did not significantly alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of any drugs (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the additional presence of indomethacin (Table 1). The absolute value of the S-I efflux for the first period of stimulation (S_1) was not significantly altered when indomethacin was present together with perindoprilat and phosphoramidon (Table 2). Similarly, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was also not significantly altered by the additional presence of indomethacin (Figure 3a).

When indomethacin (10 μM) was present together with perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin (1 μM) still reduced the S-I efflux (Figure 3a) and enhanced the resting efflux (Table 1).

Effects of bradykinin in the presence of indomethacin and N^G-nitro-L-arginine The nitric oxide synthase inhibitor N^G-nitro-L-arginine (NOLA) was used to determine whether the inhibitory effect of bradykinin on S-I efflux was due to the liberation of nitric oxide. NOLA (100 μM) and indomethacin (10 μM), when introduced together with perindoprilat (10 μM) and phosphoramidon (1 μM), did not significantly alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of any drugs (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the additional presence of NOLA (Table 1). The absolute value of the S-I efflux for the first period of stimulation (S_1) was not significantly altered when NOLA and indomethacin were present together with perindoprilat and phosphoramidon (Table 2). Similarly, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly altered by the additional presence of NOLA (Figure 3a).

When NOLA (100 μM) and indomethacin (10 μM) were present together with perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin (1 μM) still reduced the S-I efflux (Figure 3a) and did not significantly alter the resting efflux (Table 1).

Resting and S-I effluxes from epithelium-denuded tracheal preparations

Control experiments In control experiments with epithelium-denuded tracheal preparations, the mean absolute resting efflux of radioactivity for the first period of stimulation (R_1) was 2778 ± 94 d.p.m. per 2 min period ($n=42$). As with epithelium-intact preparations, there was a progressive decline in the resting efflux between the first and second periods of stimulation, thus, the mean value of the resting efflux (% R_2/R_1) was $82.2 \pm 2.6\%$ ($n=6$). The mean absolute efflux of radioactivity evoked by the first period of stimulation (S_1) was 3840 ± 318 d.p.m. ($n=42$) and the mean value of the S-I efflux (% S_2/S_1) was $96.1 \pm 5.6\%$ ($n=6$).

Effects of UK14304 and idazoxan UK 14304 (0.1 and 1 μM), introduced 15 min before the second period of stimulation, inhibited the S-I efflux in a concentration-dependent manner (Figure 1b). As with epithelium-intact preparations, the higher concentration of UK14304 (1 μM) slightly enhanced the resting efflux (Table 3).

Idazoxan (0.3 μM), when introduced 30 min before the first period of stimulation, did not alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of idazoxan (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered when idazoxan was present (Table 3). Similarly, the absolute mean value of the S-I efflux for the first period of stimulation (S_1) and the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), were not significantly altered when idazoxan was present (Table 2, Figure 1b, respectively).

Similar to the finding with epithelium-intact preparations, the inhibition of the S-I efflux produced by UK 14304 (1 μM) was antagonized by idazoxan (0.3 μM) (Figure 1b). Moreover, in the presence of idazoxan, UK14304 (1 μM) did not alter the resting efflux (Table 3). As in epithelium-intact tracheal preparations, when introduced alone 15 min before the second

Table 3 Effects of drugs on the resting efflux of radioactivity from epithelium-denuded preparations of rat isolated trachea previously incubated with [^{3}H]-choline

*Drug**	*Resting efflux (%R_2/R_1)***	*n*
None		
Control	82.2±2.6	6
UK14304 (0.1 μM)	87.7±2.3	4
UK14304 (1 μM)	96.2±2.9†	4
Idazoxan (0.3 μM)	77.4±4.7	4
Angiotensin II (0.1 μM)	99.9±2.5†	4
Angiotensin II (1 μM)	96.2±2.2†	4
Perindoprilat (10 μM)	90.7±1.2	4
Bradykinin (0.01 μM)	100.1±3.3†	4
Bradykinin (0.1 μM)	104.5±3.4†	4
Bradykinin (1 μM)	112.4±1.1†	4
Idazoxan (0.3 μM) present throughout		
Control	86.6±1.6	5
UK14304 (1 μM)	91.4±4.4	4
Angiotensin II (0.1 μM)	90.4±1.9	5
Angiotensin II (1 μM)	103.8±7.3†	4
Perindoprilat (10 μM) present throughout		
Control	86.1±1.7	4
Angiotensin II (1 μM)	103.0±4.2‡	4
Perindoprilat (10 μM)+Phosphoramidon (1 μM) present throughout		
Control	80.6±1.7	4
Bradykinin (1 μM)	120.7±2.3†	5
Perindoprilat (10 μM)+Phosphoramidon (1 μM)+indomethacin (10 μM) present throughout		
Control	86.0±1.9	4
Bradykinin (1 μM)	118.7±4.9†	4
Perindoprilat (10 μM)+Phosphoramidon (1 μM)+indomethacin (10 μM)+N^G-nitro-L-arginine (100 μM) present throughout		
Control	85.3±1.2	4
Bradykinin (1 μM)	109.4±1.5†	4

*See text for details of drug exposure. **Resting efflux for the second period of stimulation was expressed as a percentage of that for the first period of stimulation. Values are mean±s.e.mean. †Significantly different from corresponding control value ($P<0.05$, ANOVA, Dunnett's test).

period of stimulation, idazoxan (0.3 μM) did not alter either the resting efflux (Table 3) or the S-I efflux (Figure 1b).

Effects of angiotensin II and perindoprilat As with epithelium-intact tracheal preparations, angiotensin II (0.1 and 1 μM), introduced 15 min before the second period of stimulation, slightly enhanced the resting efflux (Table 3) but did not significantly alter the S-I efflux (Figure 2b).

Perindoprilat (10 μM), when introduced 30 min before the first period of stimulation, slightly enhanced the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of perindoprilat (Table 2). As with epithelium-intact preparations, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered when perindoprilat was present (Table 3). The absolute value of the S-I efflux for the first period of stimulation (S_1) was significantly greater when perindoprilat was present (Table 2). However, the mean value for the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly different when perindoprilat was present (Figure 2b).

Similar to epithelium-intact preparations, in the presence of perindoprilat (10 μM), angiotensin II (1 μM) did not alter the S-I efflux (Figure 2b) but enhanced the resting efflux (Table 3). As in epithelium-intact tracheal preparations, when introduced alone 15 min before the second period of stimulation, perindoprilat (10 μM) did not significantly alter either the resting efflux (Table 3) or the S-I efflux (Figure 2b).

Effects of angiotensin II after blockade of prejunctional α_2-adrenoceptors When introduced 15 min before the second period of stimulation, angiotensin II (0.1 and 1 μM), in the presence of idazoxan (0.3 μM), enhanced the resting efflux (Table 3) but did not alter the S-I efflux (Figure 2b).

Effects of bradykinin before and after inhibition of ACE and NEP Bradykinin (0.01 – 1 μM), introduced 15 min before the second period of stimulation, significantly reduced the S-I efflux (Figure 3b) and enhanced the resting efflux (Table 3).

As with epithelium-intact preparations, the combination of perindoprilat (10 μM) and phosphoramidon (1 μM), when added 30 min before the first period of stimulation, slightly enhanced the absolute resting efflux for the first period of stimulation (R_1), compared to that in their absence (Table 2). However, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the combined presence of perindoprilat and phosphoramidon (Table 3). The absolute value of the S-I efflux for the first period of stimulation (S_1) was not significantly altered when perindoprilat and phosphoramidon were present together (Table 2). Moreover, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly different when perindoprilat and phosphoramidon were present in combination (Figure 3b).

In the combined presence of perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin (1 μM) still reduced the S-I efflux (Figure 3b) and enhanced the resting efflux (Table 3).

Effects of bradykinin in the presence of indomethacin Indomethacin (10 μM), when introduced together with perindoprilat (10 μM) and phosphoramidon (1 μM), slightly enhanced the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of any drugs (Table 2). However, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the additional presence of indomethacin (Table 3). The absolute value of the S-I efflux for the first period of stimulation (S_1) was significantly enhanced when indomethacin was present together with perindoprilat and phosphoramidon (Table 2). However, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not altered by the additional presence of indomethacin (Figure 3b).

When indomethacin (10 μM) was present together with perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin (1 μM) still reduced the S-I efflux (Figure 3b) and enhanced the resting efflux (Table 3).

Effects of bradykinin in the presence of indomethacin and N^G-nitro-L-arginine NOLA (100 μM) and indomethacin (10 μM), when introduced together with perindoprilat (10 μM) and phosphoramidon (1 μM), did not significantly alter the absolute resting efflux for the first period of stimulation (R_1), compared to that in the absence of any drugs (Table 2). Moreover, the mean value of the resting efflux for the second period of stimulation (% R_2/R_1) was unaltered by the additional presence of NOLA (Table 3). The absolute value of the S-I efflux for the first period of stimulation (S_1) was significantly enhanced when NOLA and indomethacin were present together with perindoprilat and phosphoramidon (Table 2). However, the mean value of the S-I efflux for the second period of stimulation, expressed as a percentage of that for the first (% S_2/S_1), was not significantly altered by the additional presence of NOLA (Figure 3b).

When NOLA (100 mM) and indomethacin (10 μM) were present together with perindoprilat (10 μM) and phosphoramidon (1 μM), bradykinin (1 μM) still reduced the S-I efflux (Figure 3b) and enhanced the resting efflux (Table 3).

Discussion

This study investigated the interaction of the renin–angiotensin system (RAS), bradykinin and sympathetic nerves with cholinergic transmission in the rat airways. Experiments were conducted on epithelium-intact and epithelium-denuded preparations of rat trachea which had been incubated with [^{3}H]-choline to incorporate [^{3}H]-acetylcholine into the cholinergic transmitter stores. Under the conditions employed, the stimulation-induced (S-I) efflux of radioactivity may be taken as an index of transmitter acetylcholine release (Fabiani *et al*., 1996).

Several investigators have reported that prejunctional inhibitory α_2-adrenoceptors are present on cholinergic nerves in various airway preparations including; equine (Yu *et al*., 1993; Zhang *et al*., 1995), guinea pig (Thompson *et al*., 1990, 1992) and human (Grundstrom & Andersson, 1985). In this study, the selective α_2-adrenoceptor agonist UK14304 significantly reduced, in a concentration-dependent manner, the S-I efflux in both epithelium-intact and epithelium-denuded tracheal preparations. Moreover, the inhibition of S-I efflux produced by UK14304 was markedly antagonized by idazoxan, a selective α_2-adrenoceptor antagonist. These findings indicate that cholinergic nerves of the rat airways are also endowed with α_2-adrenoceptors which subserve inhibition of transmitter acetylcholine release. These findings are consistent with previous studies and provide further evidence of the existence of prejunctional inhibitory α_2-adrenoceptors on airway parasympathetic nerves.

It is noteworthy that the inhibitory effect of UK14304 was apparently greater in epithelium-denuded preparations as compared to epithelium-intact preparations. It is possible that the epithelium may play a permissive role in the modulatory actions of UK14304 on transmitter acetylcholine release. The airway epithelium has been shown to exert an inhibitory influence on the release of transmitter acetylcholine by a mechanism which may involve a putative inhibitory factor (Wessler *et al*., 1990, 1991). It is possible therefore that, in epithelium-intact preparations, UK14304 and the putative epithelium-derived inhibitory factor may modulate the release of transmitter acetylcholine through a similar effector pathway, such that, there is convergence in their mechanisms. Alternatively, the epithelium may simply act as a physical barrier impeding the accessibility of UK14304.

Surprisingly, idazoxan alone was without effect on the S-I efflux in either epithelium-intact or epithelium-denuded tracheal preparations. This lack of effect of idazoxan suggests that in rat airways sympathetic nerves do not modulate the release of transmitter acetylcholine although inhibitory α_2-adrenoceptors are functionally active on airway parasympathetic nerves. Since the airways of rats are known to be sparsely innervated with sympathetic nerves (Barnes, 1986, 1992), it is possible that sympathetic nerves do not lie in close proximity to parasympathetic nerves to engage in transneuronal modulation. The endogenous ligand for prejunctional α_2-adrenoceptors on airway parasympathetic nerves remains obscure but may represent sites of action for circulating catecholamines (Jones *et al*., 1980; Martin & Collier, 1986; Zhang *et al*., 1995).

Previous functional studies have suggested that angiotensin II may have prejunctional actions on cholinergic transmission (see Introduction). More direct evidence has been provided by Barnes *et al*. (1989, 1990, 1992) who showed that angiotensin II inhibits potassium-evoked release of [^{3}H]-acetylcholine from rat entorhinal cortex and human temporal cortex. In the present study, angiotensin II was without effect on the S-I efflux in both epithelium-intact and epithelium-denuded preparations of radiolabelled rat trachea. It is well established that many tissues are capable of generating angiotensin II locally (Dzau, 1987, 1988, 1989; Campbell, 1987). It is conceivable that the effects of exogenous angiotensin II may not be readily observable due to the possible influence of locally formed endogenous angiotensin II. However, this possibility would appear unlikely as angiotensin II, in the presence of the angiotensin-converting enzyme (ACE) inhibitor perindoprilat to block endogenous production of angiotensin II, was also without effect on the S-I efflux in both epithelium-intact and epithelium-denuded tracheal preparations. Moreover, perindoprilat alone had no effect on the S-I efflux in both tracheal preparations.

It is generally accepted that in dually innervated tissues such as the heart noradrenaline released from adjacent sympathetic nerves has the capacity to modulate transmitter acetylcholine release via prejunctional α_2-adrenoceptors on cholinergic nerves (Loiacono & Story, 1986; Fabiani & Story, 1995; Rand *et al*., 1990; Fuder & Muscholl, 1996). Furthermore, it has recently been shown in our laboratory that angiotensin II can facilitate the release of [^{3}H]-noradrenaline from sympathetic nerves of the rat trachea (Boicos *et al*., 1996). The possibility was considered that possible enhancement of noradenaline release by angiotensin II may modulate transmitter acetylcholine release subserved by prejunctional inhibitory α_2-adrenoceptors. However, in the presence of the α_2-adrenoceptor antagonist idazoxan to exclude possible involvement of transneuronal modulation, angiotensin II was also without effect on the S-I efflux from either epithelium-intact or epithelium-denuded tracheal preparations.

It is apparent that angiotensin II does not directly modulate the release of acetylcholine from parasympathetic nerves within the airways. This lack of effect of angiotensin II on S-I efflux is in marked contrast to the findings of Barnes *et al*. (1989, 1990, 1992) who reported an inhibitory action of angiotensin II on [^{3}H]-acetylcholine release from cholinergic nerves in the brain. This discrepancy between our findings and those of Barnes *et al*. (1989, 1990, 1992) is difficult to reconcile but may reflect differences between cholinergic nerves in the brain with those in the trachea. The findings suggest that prejunctional angiotensin II receptors are not present on cholinergic nerve terminals in the airways, or if they are present, they do not play a functional role in modulating transmitter acetylcholine release.

It has been reported that bradykinin enhances the release of [^{3}H]-acetylcholine from cholinergic nerves in the guinea pig myenteric plexus (Yau *et al*., 1986). Moreover, Omini *et al*. (1989) postulated that bradykinin may potentiate parasympathetic activity by increasing neurotransmitter release from vagal nerves. In the present study, bradykinin inhibited the S-I efflux in epithelium-denuded tracheal preparations but not epithelium-intact preparations, although there was a tendency to do so. However, bradykinin is a substrate for the degradative enzymes ACE and neutral endopeptidase (NEP) (Miura *et al*., 1992). Indeed, Rump *et al*. (1995) recently showed that the effects of exogenous bradykinin on sympathetic transmission in human kidney slices were only manifest after inhibition of ACE, suggesting that exogenous bradykinin may be rapidly broken down by this enzyme. In the present study, when ACE and NEP were inhibited by perindoprilat and phosphoramidon, respectively, to prevent substrate degradation, bradykinin inhibited the S-I efflux in epithelium-intact preparations as well as epithelium-denuded preparations. This suggests that in, epithelium-intact preparations, exogenous bradykinin is rapidly broken down by epithelial ACE and NEP such that the effects of bradykinin on cholinergic transmission are not apparent. However, when degradation of bradykinin is prevented, either by inhibiting epithelial ACE and NEP or removing the epithelium, the inhibitory effects of bradykinin on cholinergic transmission in the airways are manifested. This inhibitory effect of bradykinin on transmitter release is in contrast to the findings of Yau *et al*. (1986) and does not lend support to the proposal by Omini *et al*. (1989) that bradykinin may enhance the release of acetylcholine from parasympathetic nerves in the airways.

It has been shown that bradykinin can stimulate the release of prostaglandin E_2 from cultured canine airway epithelial cells which inhibits smooth muscle contraction induced by electrical field stimulation (Barnett *et al*., 1988). It was concluded that prostaglandin E_2 acts at a prejunctional site to inhibit neuro-

transmitter release from cholinergic nerves. However, in our study, the inhibition of S-I efflux produced by bradykinin in both epithelium-intact and epithelium-denuded preparations was unaffected by the cyclo-oxygenase inhibitor indomethacin. Schlemper & Calixto (1994) recently reported that the relaxant response of bradykinin in epithelium-intact preparations of guinea pig trachea is mediated by nitric oxide. The possibility was considered that the inhibitory effects of bradykinin on the S-I efflux might be due to a mechanism involving the release of nitric oxide. However, the inhibitory effect of bradykinin on S-I efflux in both epithelium-intact and epithelium-denuded preparations was unaltered by the additional presence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine. Taken together, these findings suggest that the inhibitory action of bradykinin on cholinergic transmission in the rat airways does not involve release of prostaglandins and/or nitric oxide.

In conclusion, the findings of the present study suggest that airway parasympathetic nerves are endowed with α_2-adrenoceptors which subserve inhibition of transmitter acetylcholine release. Under the present conditions however, transmitter acetylcholine release is not subject to transneuronal modulation by noradrenaline released from adjacent sympathetic nerves in the airways. Moreover, angiotensin II and perindoprilat do not appear to modulate acetylcholine release from parasympathetic nerves of the airways. In contrast, bradykinin inhibits acetylcholine release from airway parasympathetic nerves but this action of bradykinin is limited by the activity of epithelial angiotensin-converting enzyme and/or neutral endopeptidase. The inhibitory action of bradykinin on cholinergic transmission in the airways does not appear to involve the liberation of prostaglandins or nitric oxide.

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