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Contractile activity of calcitonin gene-related peptide on pulmonary tissues

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Abstract—Rat calcitonin gene-related peptide was shown to be a very potent contractile agent on guinea-pig trachea, contractions being observed at 10 pM and maximal contractions (32–36% of the methacholine maximum) being observed between 0·3 and 13·3 nM. The response was largely inhibited (88%) by pretreatment with indomethacin indicating that the effect was mediated through the secondary generation of cyclooxygenase products.

Calcitonin gene-related peptide (CGRP) is a potent vasodilator agent that is present with substance P in the afferent nerves of various organs including the lungs (Goodman & Iversen 1986). In addition to its vasodilator activity, rat CGRP (rCGRP) has been shown to have potent contractile activity on guinea-pig gastrointestinal smooth muscle preparations (Ghatei et al 1984; Tippins et al 1984). On human bronchi, CGRP was a potent spasmogenic agent and the response was not inhibited by various blockers suggesting a direct effect of CGRP on human respiratory smooth muscle (Palmer et al 1987). Accordingly in the present study we have examined the contractile response to rCGRP on pulmonary smooth muscle preparations from the guinea-pig and the rat.

Methods

Tracheal chains and parenchymal strips from guinea-pigs and inbred hyperreactive rats (Brunet et al 1983) were prepared according to the method of Jones et al (1982) and suspended under 1 g of tension in tissue baths containing modified Krebs buffer at 37°C and gassed with 95% O_2 and 5% CO_2. Isometric responses were recorded and the contractions calculated as percentages of the methacholine ($1{\cdot}5 \times 10^{-5}$ M) maximum and are shown as means ± s.e.m.

Results

Addition of rCGRP (Sigma) in solution in distilled water to the guinea-pig isolated trachea produced a contraction that was rapid, dose-dependent, long lasting and easily reversed by washing. The rCGRP threshold dose was above 10 pM and the maximum contraction (31·8 ± 10·5%, n = 11) following cumulative addition was achieved between 3·3 and 13·3 nM. From the cumulative dose response curve an EC50 of 0·46 ± 0·19 nM (mean ± s.e.m., n = 11) was calculated by linear regression analysis. A different degree of activity was observed if rCGRP was administered as a single dose per tissue. Under these conditions rCGRP induced a contraction which was maximal at 0·3 nM (36·6 ± 4·9% n = 14). In a separate experiment 0·3 nM rCGRP was applied twice to the same tissue 60 min apart and no tachyphylaxis was observed (1st response: 46·6 ± 8·5 vs 2nd response: 60·6 ± 14·0%, n = 3). Under these conditions pretreatment of the tissues with the cyclooxygenase blocker, indomethacin (5·6 μM) markedly inhibited (88·1 ± 5·9%, n = 8, $P > 0{\cdot}001$, paired t-test) the response to 0·3 nM rCGRP. The response to rCGRP was not affected by the addition of a mixture of peptidase inhibitors, bacitracin (5 μg mL^{-1}), leupeptin (1 μg mL^{-1}) and chymostatin (1·25 μg mL^{-1}). The peptide (1 pM–1 mM) was also tested on guinea-pig parenchyma, rat trachea and rat parenchyma and had no contractile effect in the presence or absence of peptidase inhibitors on these tissues, suggesting that proteolytic breakdown is not modifying the action of rCGRP on the various tissues.

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Discussion

The present data would suggest that rCGRP is one of the most potent contractile agents on guinea-pig trachea yet described indicating the presence of high affinity receptors to CGRP on this tissue. The in-vitro contractile activity of CGRP is not consistent with an apparent lack of activity in the respiratory system when administered i.v. to the guinea-pig (Lundberg et al 1985). However, the peptide may produce different responses when administered by the aerosol route and has been reported for leukotriene D_4 in the rat (Brunet et al 1983). In contrast to the activity of trachea, the peptide had no contractile activity on either the guinea-pig parenchyma or the rat trachea or parenchyma. The potency of the peptide on the guinea-pig trachea was similar to that reported on guinea-pig gastrointestinal tissues (Ghatei et al 1984; Tippins et al 1984) and somewhat higher than that observed on human bronchi (Palmer et al 1987). On human bronchi, CGRP contracted the tissue through a direct activation of a specific receptor. In contrast, the contraction induced by rCGRP on the guinea-pig trachea was markedly inhibited by indomethacin, indicating that synthesis and release of cyclooxygenase products were involved in the response. In this respect the guinea-pig trachea differs from the human bronchi and rat trachea with regard to the contribution of cyclooxygenase products to the intrinsic tone and the contractile processes of certain spasmogens (Lawson et al 1986). The release of cyclooxygenase products by rCGRP on guinea-pig trachea may reflect a limited activation of phospholipase C during a secondary transduction process and hence maximal contractions were not observed. The role of CGRP receptors on respiratory tissues may relate to other physiological process and this may explain the inactivity of rCGRP on rat respiratory tissues despite the fact that CGRP has been associated with neurons in rat lung (Goodman & Iversen 1986). Receptor binding and further functional studies should reveal the role of the CGRP in the respiratory tract.

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Kinetic mechanism for the intestinal absorption of ofloxacin

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Abstract—The absorptive behaviour of ofloxacin, a quinolone antibacterial agent, was studied following recirculation in small intestine of both male and female rats, at initial doses ranging from 0·125 to 5 mg mL^{-1}. A saturable Michaelis-Menten process is suggested to explain the intestinal absorption. No significant differences were found in the absorption parameters per metabolic weight unit.

Drug absorption through the small intestine is the result of passage across a cellular layer—the brush-border epithelium of intestinal villi. Overton's first rule assumes that the permeability of membranes to small molecules is directly related to their lipid solubility. According to Schanker et al (1958), the degree of ionization of a drug also conditions its absorption, since the oil/water partition coefficient is higher in the un-ionized than in the ionized state of a drug. If a drug is not liposoluble enough to pass through cell membranes by simple diffusion, carrier proteins may improve the absorption. This process fits Michaelis-Menten saturating kinetics and may not require metabolic energy.

Studies on drug absorption kinetics are often aimed at determining apparent absorption rate constants (k_{app}) for a series of initial concentrations of a drug as indicators for its disappearance from the intestinal lumen with time. The constants should include both true absorption and degradation rate constants for the overall process and can be used as starting point for the estimation of true kinetic parameters such as carrier affinity or maximum absorption rate.

The 4-quinolone antibiotic group has a wide antibacterial spectrum, both in-vitro and in experimental infective processes, which resembles the activity of β-lactam antibiotics and aminoglycosides (Neu & Labthavikul 1982). Although the mechanism of the quinolones' antibacterial actions is not yet fully understood, it may be possible that the effect lies in the inhibition of bacterial gyrase enzyme, there by inducing death of the microorganism (Smith 1983).

Previously Prieto et al (1987), observed no significant changes in the intestinal absorption of a second-generation quinolone, ofloxacin DL-8280, in male and female rats, as opposed to findings with other drugs (Foradada et al 1974). We have now attempted to ascertain from among the possible mechanisms of intestinal absorption whether free diffusion and saturating transport are involved in the uptake of ofloxacin.

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Materials and methods

Adult Wistar rats of either sex were used. Body weight was measured twice weekly and gave a sigmoid growth curve (Hammond 1932) similar to other species. Previous data on growth (Prieto et al 1987) showed no significant differences in body weight between male and female rats at weaning, but significant differences in the plateau region of the growth curve as well as the growth rate from the weaning to the adult state. The average values with coefficient of variation were:

Initial weight: males 41·79CV32·28, females 39·25CV35·73. (NS) and
final weight: males 331·52CV22·02, females 263·38CV18·46 ($P < 0·001$).

Perfusion technique. Animals were anaesthesized using urethane (1·5 kg^{-1}) 1 h before starting the experiments. A recirculating perfusion technique was used (Tsuji et al 1978;Ponz et al 1980). An intestinal length of segment of 20 cm taken from the pyloric sphincter was cannulated after ligation of the bile duct, washed with 50 mL of drug-free buffer solution, and perfused with drug solution at known initial concentration, both solutions being pre-heated to 37°C and maintained at this temperature. The perfusion rate was 2 mL min^{-1}. Successive samples were taken at 5 min intervals for 90 min during recirculation of the drug solution. The perfusing medium consisted of 0·05 M phosphate buffer pH 7·4 with ionic strength adjusted to 0·15 by sodium sulphate. In all experiments 50 mL of the different drug concentrations in this buffer solution were perfused.

High-performance liquid chromatography (HPLC) was used to analyse the ofloxacin content of the samples, from which the apparent absorption rate of the drug was assessed. Three males and three females were used in the corresponding perfusion experiments for each one of seven ofloxacin concentrations.

In the HPLC analysis, a low-polarity mobile phase (hydro-alcoholic mixture 90:2:8 (v/v of methanol,—0·05 M phosphate buffer pH 6·0—water) was used. The stationary phase consisted of a polar 5-CN-group column.

Results and discussion

Recirculation of different initial doses of ofloxacin (0·125 to 5·000 mg mL^{-1}) gave results corresponding to the ofloxacin concentration remaining unabsorbed with time. Linear regression of the natural logarithm of concentration versus time gave slope values of the apparent absorption rate constants (K_{app}, min^{-1}).