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The presence of mucosa reduces the contractile response of the guinea-pig urinary bladder to substance P

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The contractile response to substance P in-vitro is greater in strips of guinea-pig bladder freed of mucosa than in normal strips, while the response to field stimulation, histamine or KCl is unaffected by the presence of mucosa. Substance P has no inhibitory effect on histamine-induced contractions of the guinea-pig bladder. These findings support the possibility that the presence of mucosa may reduce accessibility of substance P to the muscle layer.

Multiple receptors mediate the biological effects of tachykinins in various tissues (Lee et al 1982; Buck et al 1984; Regoli et al 1985). Owing to lack of potent and selective antagonists, determination of the relative order of potency of tachykinins in producing a given effect (Lee et al 1982; Regoli et al 1985, 1986) or in displacing selective ligands in bindings assays (Buck et al 1984; Burcher & Buck 1986) is the most important criterion for classifying tachykinin receptors. Functional studies on selected preparations (dog carotid artery, rabbit pulmonary artery and rat portal vein) have indicated the existence of at least three tachykinin receptors in mammalian tissues (Regoli et al 1985, 1986). However, in most preparations, the rank order of potency of agonists in producing a given effect does not exhibit a clear pattern indicative of the presence of one receptor. This occurs for a variety of reasons among which a prominent place is ascribable to the fact that multiple tachykinin receptors are often present in the same tissue where they may mediate: (i) the same type of final response (see Maggi et al 1986a), (ii) different components of a similar response (such as neurogenic and myogenic components of contractions) (see Maggi et al 1986b), or (iii) opposite effects on the parameter under study (see D'Orleans-Juste et al 1986).

We now present evidence that the presence of certain tissue components may influence responsiveness of other parts of the preparation to exogenous substance P, thus reinforcing the concept that great care is needed when aiming to classify tachykinin receptors on the basis of functional data.

Materials and methods

Male albino guinea-pigs, 200–260 g, were stunned and bled. The whole urinary bladder was rapidly removed and placed in standard Krebs solution gassed with 96% O_2 plus 4% CO_2, as described previously (Maggi et al 1985). The bladder base (a circular strip taken just below and above the ureters and comprising the ureterovesical junction) was excised from the bladder body and urethra. From each bladder base two circularly oriented strips were dissected: in one of them the mucosa was gently and carefully removed and the strips were placed in a 5 mL organ bath at 37 °C. The strips were connected to an isometric strain gauge under a load of 1 g and field-stimulated (0·1 Hz, 0·5 msec, 60 V) by means of two platinum wire electrodes placed at the top and the bottom of the organ bath. When the response to field stimulation had reached a steady state, (about 60 min) the contractile response to substance P was established by constructing a non-cumulative concentration-response curve at 15–20 min intervals between doses. At the end of the experiments the strips were exposed to histamine (0·3 mM) and KCl (100 mM) to determine their maximal response. Then the strips were blotted two or three times on filter paper and weighed. The strips containing the mucosa were weighed before and after its removal. The mucosa accounted for 20–30% of wet weight of the strips. The spontaneous activity of the strips or the contractile response to field stimulation, histamine, KCl or substance P were expressed either as mg of contraction per mg of wet weight (muscosa free) or as % of response to KCl. Regression analysis was performed by means of the least squares method. EC50 and 95% confidence limits were calculated accordingly.

Statistical analysis of the data was performed by means of Student's *t*-test for unpaired data. Each value is mean ± s.e. of the mean. Substance P and rat calcitonin gene-related peptide (CGRP) were from Peninsula, histamine 2HCl was from Carlo Erba.

Results

The amplitude of spontaneous contractions was greater in mucosa-free (8·5 ± 1 mg mg^{-1} of wet weight) than control strips (2·8 ± 0·8, n = 6 for each group, $P < 0·01$) while frequency was similar in both groups (7·3 ± 1 and 6 ± 1 contractions min^{-1}, respectively, n.s.). Response to field stimulation, histamine or KCl was unaffected by removal of mucosa (see Table 1) while maximal response to substance P (3 μM) was lower in the presence of mucosa. Further experiments revealed that both threshold concentration (10 and 30 nM in the absence and presence of mucosa, respectively) and sensitivity to substance P were affected by the presence of mucosa. Mucosa-free preparations were slightly

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Table 1. Contractile responses to substance P, KCl, histamine or field stimulation of guinea-pig bladder strips in the presence and the absence of mucosa.

Stimulus	Concn	Contractile response (mg × mg of wet weight) With mucosa	Without mucosa
Substance P	3 μM	22 ± 2	64 ± 6*
KCl	80 mM	115 ± 18	129 ± 25
Histamine	0·3 mM	182 ± 19	219 ± 72
Field stimulation	—	56 ± 5	60 ± 9

Each value is mean ± s.e. of the mean of at least 4 experiments. *$P < 0.01$.

more sensitive and developed stronger responses than preparations with intact mucosa (Fig. 1). EC50 and 95% c.l. (in brackets) were 61 (38–110) and 116 nM (101–135) in the absence and presence of mucosa, respectively.

The question was raised as to whether the lower response to substance P in the presence of mucosa may be influenced by the production of some factor having an inhibitory effect on motility. To explore this point we studied the effect of substance P (10–100 nM) on histamine-induced rhythmic bladder contractions. After an initial large contractile response (data shown in Table 1), histamine induced a series of high-amplitude (86 ± 25 and 54 ± 15 mg mg^{-1} of wet weight in the absence and presence of mucosa, n = 4 for each group respectively, n.s.) and low-frequency (1–3 contractions min^{-1}) rhythmic contractions which lasted for at least 30 min. Addition of substance P (10–100 nM) had no inhibitory effect on these rhythmic contractions which, however, were promptly suppressed by rat CGRP (0·1 μM) both in the presence and the absence of the mucosal layer (n = 4 for each group).

Discussion

The mechanical response of the guinea-pig bladder to substance P and other tachykinins was investigated by a number of authors; some of them used mucosa-free detrusor strips (Mackenzie & Burnstock 1984; Hourani 1984), while in other studies no details about this point were provided (Growcott et al 1983; Watson et al 1983). Our findings indicate that the presence of mucosa markedly reduced amplitude of substance P but not histamine-, KCl- or field stimulation-induced contractions of isolated strips from the guinea-pig bladder base. The observation that sensitivity to substance P was slightly greater in the absence than in the presence of mucosa may suggest that, in normal strips, substance P is metabolized at a faster rate and/or its diffusion at receptor sites in the muscle is delayed, thus favouring its catabolism.

This hypothesis is further supported by preliminary observations indicating that the response of the guinea-pig bladder base to neurokinin B was unaffected by removal of mucosa (M. Parlani and M. Astolfi, unpublished data).

Irrespective of the mechanisms involved, our findings demonstrate that the presence of certain tissue components (mucosa) may influence the response of other ones (muscle) to substance P. This possible drawback should be considered when analysing the effect of agonists (tachykinins) in producing a biological effect in terms of receptor subtypes involvement.

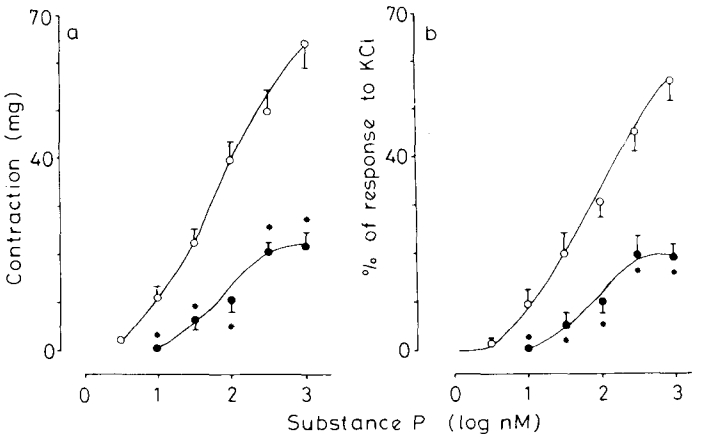

FIG. 1. Contractile response to substance P in bladder strips of the guinea-pig urinary bladder either in the presence (●) or the absence (○) of mucosa. Each value is mean ± s.e. of 6 experiments. * Significantly different from the corresponding value obtained in mucosa-free strips, $P < 0.01$.

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The biliary excretion of acenocoumarol in the rat: stereochemical aspects

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Within 24 h, 50% of a single dose of the acenocoumarol enantiomers was recovered in bile and 20% in urine of Wistar rats. The elimination products were mainly (>90%) the 6- and 7-hydroxyacenocoumarol as conjugates in the bile but free in the urine. Only *R*-acenocoumarol, free and conjugated, was excreted in bile. There were no gross differences between the enantiomers in metabolic pattern or in the amount of metabolites formed. A significant difference was observed for the biliary excretion of the 7-hydroxy metabolite; the ratio of free and conjugated 7-hydroxyacenocoumarol was three times higher for the *S*- than for the *R*-isomer. An unknown third metabolite was recovered in bile in higher amounts with the *S*- than with the *R*-acenocoumarol. Only traces of this metabolite were recovered from urine. The data show an extensive biliary excretion of acenocoumarol and demonstrate stereoselective mechanisms in the excretion processes.

The enantiomers of the 4-hydroxycoumarin acenocoumarol differ in their pharmacokinetics. In the rat, the *S*-enantiomer is cleared four times faster (Thijssen et al 1985), and in man 10 times faster (Godbillon et al 1981; Thijssen et al 1986), than the *R*-isomer. As the drug is eliminated predominantly by biotransformation and as there is hardly any difference in plasma protein binding between the enantiomers (Thijssen et al 1985), the difference in body clearance reflects stereoselective differences in the intrinsic biotransformation rate. For the chemical congener, warfarin, stereochemical differences in the rate as well as in the route of metabolism have been demonstrated (Pohl et al 1976a, b). Little is known about the fate of acenocoumarol. We previously showed in rats that reduction of the aromatic nitro-group of acenocoumarol did not occur (Thijssen et al 1985). In man, two hydroxylated metabolites, i.e. the 6- and 7-hydroxylated derivatives (Fig. 1) were recovered from urine (Thijssen et al 1986). We have investigated the biliary and urinary excretion of single doses of the *R*- and *S*-enantiomers of acenocoumarol in the rat to find whether differences in the metabolic route between the enantiomers explain their pharmacokinetic differences.

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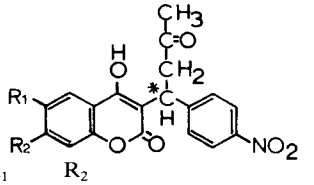
Materials and methods

The optically pure *R*- and *S*-enantiomers of acenocoumarol were a gift from Ciba-Geigy, Basel, Swizerland. Male inbred Wistar rats (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands), 300–350 g, were used. They had free access to water and food.



R_1	R_2	
H	H	acenocoumarol
OH	H	6-hydroxyacenocoumarol
H	OH	7-hydroxyacenocoumarol

FIG. 1. Structure of acenocoumarol and its 6- and 7-hydroxy metabolite.