urine. For this reason, the lipophilicity is the primary criterion affecting the renal pathway of structurally similar compounds.

For the three species studied, the values of parameters a and b in the renal clearance-lipophilicity equations are surprisingly similar, indicating no significant interspecies differences in dependency of renal clearance on lipophilicity. Even if correlation coefficients are sufficient for biological experiments, their values are not too high indicating that not only lipophilicity, but also other factors (molecular structure, pK_a, biotransformation, etc.) can affect to a certain degree the renal elimination of these compounds. Nevertheless, the approach to lipophilicity-renal clearance profiles from three different species into a unique species-independent profile by using equation 2 demonstrates the similarity of renal elimination of these organic acids among rabbits, rats, and mice, and perhaps it could provide the foundation for interspecies scaling of this route of elimination in mammals.

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Evidence against Substance P as a neurotransmitter at the neuroepithelial junction in rat colonic mucosa

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Abstract—Substance P (SP) caused a concentration-dependent increase in short-circuit current of rat isolated colonic mucosal preparations (ED50 10 nM). The SP antagonist [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (50 μM) did not increase short-circuit current. Tetrodotoxin (3·1 μM) reduced the effect of a maximum concentration of SP (300 nM). This reduction was increased when tetrodotoxin was given with [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. Increases in short-circuit current produced by electrical field stimulation were not reduced by [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. It is concluded that SP is not a transmitter at the neuroepithelial junction in rat colonic mucosa.

Substance P (SP)-like material has been detected by radioimmunoassay in the intestinal mucosa of a number of species (Keast et al 1985a). Such fibres may innervate enterocytes, blood vessels or other nerves. In-vitro studies, using intact sheets of ileal mucosa, have shown SP to produce an increase in short-circuit current (s.c.c.). This effect was mediated by a combination of actions on secretomotor neurones and enterocytes (Keast et al 1985b) or histamine-releasing cells (Kuwahara & Cooke 1990).

There is conflicting evidence as to whether SP is a secretomotor neurotransmitter in the intestine. In rabbit ileal mucosa the s.c.c. response to nerve stimulation was unaltered by desensitization to SP (Hubel 1984), whereas in guinea-pig ileal mucosa, SP desensitization, SP antibodies (Perdue et al 1987) and SP antagonists (Keast et al 1985c; Kuwahara & Cooke 1990) reduced electrically-evoked increases in s.c.c.

The purpose of the present investigation was to determine whether SP might function as a secretory neurotransmitter at the neuroepithelial junction in rat colonic mucosa.

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Methods

Segments of large bowel were obtained from male Wistar rats, 180–250 g. The segment consisted of an approximately 10 cm length extending from proximal colon to distal rectum. The segments were opened longitudinally along the anti-mesenteric border and pinned mucosa downwards in Krebs-Henseleit solution. Muscle-stripped preparations consisting of mucosa, muscularis mucosa and submucosa were prepared by dissection under a binocular microscope. The striated part of the proximal colon was not used. Mucosal sheets were mounted in Ussing chambers (0·64 cm^2 window area). Five mL of Krebs solution bathed each side of the preparation, this was reduced to 3 mL each side when using the SP antagonist.

Electrical field stimulation (1 ms, 150 pulses at 1 or 10Hz) of mucosal nerves was achieved by passing monophasic pulses across the tissue using a Grass S88 stimulator connected to two aluminium foil electrodes. One foil was placed either side of the preparation to reduce short-circuiting through the bathing fluid (Hubel, personal communication). Some tissue was fixed in 10% phosphate buffered formalin after which haematoxylin- and eosin-stained sections were prepared for histological examination. Composition of the Krebs fluid was (mM): NaCl 118, glucose 11·5, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·2, KH_2PO_4 1·2, $CaCl_2$ 2·5.

Transmucosal short circuit current was measured with a high impedance voltage clamp (DCV-1000, World Precision Instruments). Compensation was made for fluid resistance between the tips of the voltage electrodes. Both current-passing and voltage-detecting electrodes utilized a system of Ag/AgCl half cells which screw into short, large-diameter tubes filled with 4% agar in 3 M KCl. Short circuit current was continuously displayed on pen recorders. All electrical values quoted are calculated for an

exposed membrane area of 1 cm^2. SP and the SP antagonist were added only to the basolateral side of the membrane. All other drugs were added to both sides. A contact time of 15 min was allowed for all antagonist drugs.

Results are expressed as mean ± s.e.m. Statistical differences between means was assessed using the Mann-Whitney U-test for unpaired data. A probability value < 0.05 was taken to represent a significant difference. Drugs used were: substance P, [D-Arg^1 D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP, (SP antagonist, Cambridge Research Biochemicals) mepyramine maleate (May & Baker), ranitidine hydrochloride (Glaxo), hyoscine hydrobromide (Sigma) and tetrodotoxin (Sigma).

Results

Histological examination of the tissue preparation confirmed the presence of mucosa, muscularis mucosa and submucosa. Electrical field stimulation (EFS) of mucosal preparations gave voltage dependent (2·5–40 V) increases in s.c.c. ranging from 3·5 ± 1·0 to 68·8 ± 7·3 $\mu A\ cm^{-2}$ for 1 Hz and from 28·0 ± 2·3 to 84·8 ± 13·5 $\mu A\ cm^{-2}$ for 10 Hz. Tetrodotoxin (TTX, 3·1 μM) reduced responses to EFS (1 and 10 Hz, 20 V, n = 10) by 97·2 ± 0·7%.

SP (0·4–300 nM, n = 6) gave a concentration-dependent increase in s.c.c. which reached a maximum value of 124·2 ± 17·5 $\mu A\ cm^{-2}$, ED50 10 nM. Desensitization to SP was kept to a minimum by using a 30 min dose cycle with four changes of chamber fluid between doses. Employing this schedule, desensitization reduced the second and third responses to SP (300 nM) by 20·9 ± 5·1 and 29·0 ± 6·8% (n = 4), respectively, compared with the first response to SP. Therefore, in experiments analysing the responses to SP, drugs were given after the second response to SP and allowance made for the small degree of desensitization. Addition of SP (300 nM) to the apical (mucosal) side of the membrane produced no change in s.c.c. (n = 4).

The increase in s.c.c. due to SP (300 nM) was reduced by 67·9 ± 4·6% in the presence of TTX (3·1 μM, n = 6), submaximal increases in s.c.c. to SP (33 nM) were reduced by 91% (n = 2). [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (50 μM) plus TTX (3·1 μM) reduced responses to SP (300 nM) by 83·0 ± 2·0% (n = 7). This was significantly greater than the antagonism produced by TTX alone. Mepyramine (100 nM) plus ranitidine (1 μM) plus TTX (3·1 μM) reduced responses to SP (300 nM) by 70·5 ± 5·2% (n = 4) which was not significantly different from the antagonism obtained using only TTX.

Increases in s.c.c. produced by EFS (1 and 10 Hz, 40 V) were not reduced by 50 μM [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. This series of experiments was carried out in the presence of hyoscine (10 μM). The concentration of SP antagonist used did not produce a change in basal s.c.c. levels.

Discussion

SP increases s.c.c. across mucosal sheets from guinea-pig ileum and colon when applied to the basolateral domain (Keast et al 1985b; Perdue et al 1987; Kuwahara & Cooke 1990). Most of the response is due to stimulation of secretomotor neurones since it is blocked by TTX. The remainder may either be due to a direct action on mucosal epithelial cells (Keast et al 1985b) or an indirect action via release of histamine (Kuwahara & Cooke 1990). Using the SP antagonist [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP, Keast et al (1985b) proposed the existence of two types of SP receptor in the mucosa and submucosa of the guinea-pig small intestine. Only those receptors on mucosal epithelial cells could be blocked by [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. That the antagonist could also be shown to reduce hyoscine resistant responses to electrical field stimulation is evidence that SP is a neurotransmitter released at the neuroepithelial junction in guinea-pig ileum (Keast et al 1985c).

In rat colonic mucosa, TTX also reduced the s.c.c. response to SP. For an analysis of the TTX-resistant response a high concentration of SP was used as it was less sensitive to TTX. Although SP can release histamine (Kuwahara & Cooke 1990), the TTX-resistant response was not due to histamine release as a combination of H_1- and H_2-receptor antagonists given with TTX did not increase the antagonism seen with TTX alone. SP may have been releasing histamine but as histamine increases s.s.c. by an action on neural elements (Frieling et al 1989; Wang & Cooke 1989) its contribution to any SP response would probably have been blocked by TTX. [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP antagonized the effects of SP on mucosal epithelial receptors in the rat colon, as shown by its action on the TTX-resistant component of the response to SP. That it did not reduce the s.c.c. response to two different frequencies of electrical field stimulation is evidence against SP being a neurotransmitter released at the neuroepithelial junction in rat colonic mucosa. Species variation may explain the different results obtained using guinea-pig small intestine and rat colon.

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