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The action of R51619 on transport processes in the rat small intestine

JACQUELINE HARDCASTLE*, P. T. HARDCASTLE, DEBRA K. KELLEHER, *Department of Physiology, Sheffield University, Sheffield S1O 2TN, U.K.*

R51619, an agent reported to release endogenous acetylcholine, increased the potential difference and short-circuit current across non-stripped, but not stripped sheets of rat mid-intestine. The response was abolished by both hexamethonium and atropine. R51619 stimulated fluid accumulation by intestinal loops in-vivo suggesting its predominant effect is to stimulate anion secretion. These results are consistent with R51619 releasing endogenous acetylcholine within the myenteric plexus to activate postganglionic cholinergic fibres which stimulate intestinal secretion via muscarinic cholinoreceptors.

Although it is well established that exogenously applied acetylcholine stimulates intestinal secretion (Hardcastle & Eggenton 1973; Isaacs et al 1976), there is little evidence that endogenously released acetylcholine can activate the secretory process. R51619 (*cis*-4-amino-5-chloro-*N*-[1-[3-(4-fluorophenoxy)propy]-3-methoxy-4-piperidinyl]-2methoxybenzamide monohydrate, mol. wt = 483·97) (Janssen Pharmaceuticals) stimulates gastrointestinal motility, an effect explained by an increased release of acetylcholine from intramural cholinergic nerves (Schuurkes et al 1982). Thus, this agent may be of use in determining a possible role for endogenous acetylcholine in the regulation of gastrointestinal function. The present investigation was designed to assess the involvement of endogenous acetylcholine in the control of intestinal secretion by studying the effects of R51619 on rat small intestine.

Methods and results

The effect of R51619 on intestinal electrical activity was measured in-vitro using paired, non-stripped (muscle layers intact) sheets of rat mid-intestine as described by Hardcastle et al (1981). The addition of R51619 to the mucosal solution to give a concentration of 6×10^{-5} M caused a transient rise in potential difference (PD, $P < 0{\cdot}05$) and short-circuit current (SCC, $P < 0{\cdot}05$) with no significant ($P > 0{\cdot}05$) change in tissue resistance (Fig. 1). The vehicle used to dissolve the drug (mannitol 40 mg + acetic acid 3 mg ml^{-1} water) was without significant effect ($P > 0{\cdot}05$) on any of the indices measured (Fig. 1). The transient nature of the response could not be attributed to the breakdown of the drug since transferring the bathing solution from one sheet produced a normal response in a second sheet. The magnitude of the response to R51619 was proportional to its concentration, a sigmoid relationship between the change in SCC and log concentration being obtained. The maximum change in PD was 1.4 ± 0·2 (3) mV and in SCC was 34·4 ± 3·4 (3) μA cm^{-2} and these occurred at $1{\cdot}2 \times 10^{-4}$ M. Addition of R51619 to the serosal side of the preparation failed to elicit a response. R51619 was also without significant effect ($P > 0{\cdot}05$) on the electrical activity of stripped sheets of intestine, suggesting that its site of action had been removed with the muscle layers.

The electrical changes induced by R51619 (6×10^{-5} M) were abolished by prior addition of hexamethonium (10^{-4} M) to the bathing solutions, implicating ganglionic transmission in the response (Fig. 2). Atropine (10^{-4} M) also eliminated the response to R51619 (Fig. 2), suggesting the involvement of muscarinic cholinoreceptors. Neither hexamethonium nor atropine alone had any effect on the SCC at the concentrations used.

A rise in SCC reflects an alteration in net ion transport but it does not indicate the direction of ion movement. Increased cation absorption and enhanced anion secretion will both lead to an increased SCC. To distinguish between these two possibilities the effect of R51619 on fluid movement across the rat mid-intestine was measured in-vivo using a modification of the enteropooling assay as described by Hardcastle et al (1983). A 15 cm segment of intestine was washed out with 154 mM NaCl and any remaining fluid was gently blown out. The loop was tied off and after 15 min it was removed from the animal and its contents collected and weighed. The empty loop was also weighed so that the volume of the contents could be related to the wet weight of the loop. When R51619 was administered i.v. at a dose of 1 mg kg^{-1} to test animals, the volume of fluid accumulating in the intestinal segment (0·093 ± 0·019 (6) ml g^{-1} wet wt/15 min) was significantly greater ($P < 0{\cdot}05$) than that obtained in control animals (0·036 ± 0·012 (6) ml g^{-1} wet wt/15 min) which received an equivalent volume of vehicle. This shows that R51619 has induced a net secretion of fluid by the small intestine and this, taken in conjunction with the electrical data, suggests that the predominant effect of R51619 is to stimulate anion secretion.

Discussion

R51619 has been shown to release endogenous acetylcholine in the gastrointestinal tract (Schuurkes et al 1982) and this could explain its secretory effects in the

* Correspondence.

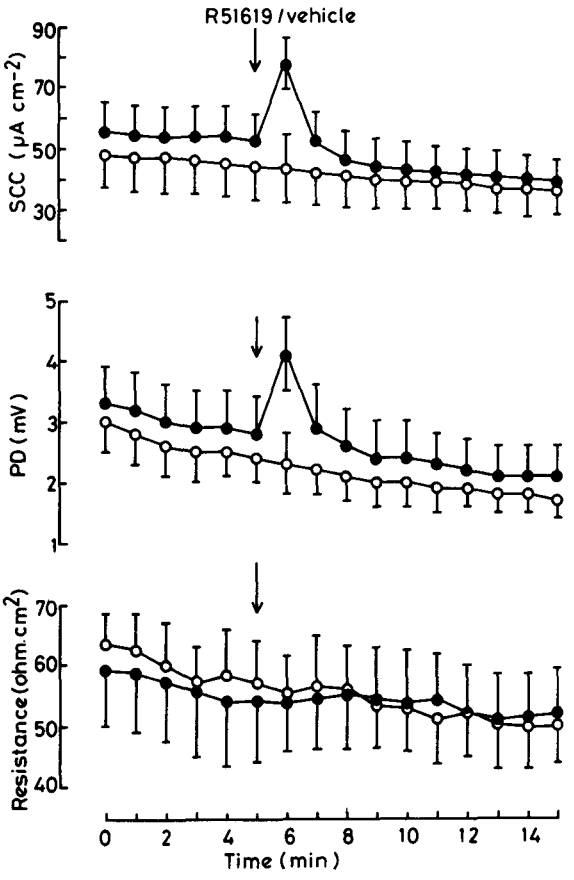

FIG. 1. The effect of adding R51619 (bath concentration 6×10^{-5} M–●) or an equivalent volume of vehicle (40 mg mannitol + 3 mg acetic acid ml^{-1} water–○) to the mucosal solution on the short-circuit current (SCC), potential difference (PD) and resistance (R) generated by intact sheets of rat mid-intestine. Each point represents the mean ± 1 s.e.m. of 4 observations.

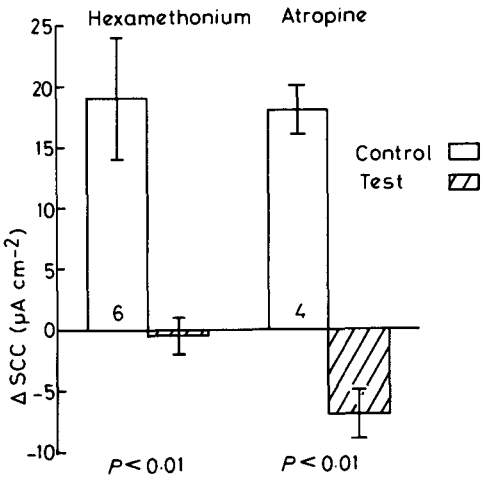

FIG. 2. The SCC changes induced by the addition of R51619 (bath concentration 6×10^{-5} M) to the mucosal solution of sheets of rat mid-intestine in the presence and absence of hexamethonium bromide (10^{-4} M in mucosal and serosal solutions) or atropine sulphate (10^{-4} M in mucosal and serosal solutions). Each bar represents the mean ± 1 s.e.m. with the number of tissue pairs indicated.

small intestine. Exogenous acetylcholine is known to induce intestinal secretion via muscarinic cholinoreceptors (Hardcastle & Eggenton 1973; Isaacs et al 1976) and the action of R51619 also appears to involve these receptors since it is inhibited by atropine. The fact that the action of R51619 is abolished by hexamethonium suggests that ganglionic transmission is involved, probably at the myenteric plexus, since removal of the muscle layers eliminated the response. R51619 could either release acetylcholine from the preganglionic nerve terminal or directly stimulate nicotinic cholinoreceptors on the postganglionic fibre. Either action could result in the activation of the postganglionic fibre and the acetylcholine released from this fibre would act on muscarinic receptors located on the enterocyte (Isaacs et al 1982) to induce anion secretion. A direct stimulation of nicotinic cholinoreceptors on the postganglionic fibre seems unlikely however, since in the chick biventer striated muscle preparation R51619 has no affinity for nicotinic receptors (Schuurkes et al 1982).

R51619 has therefore been shown to induce intestinal secretion, an effect that can be explained in terms of its ability to release endogenous acetylcholine. Thus it is possible that endogenous sources of this neurotransmitter could play a role in the regulation of secretory processes in the small intestine.

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