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Supersensitivity to morphine after chronic sympathetic denervation in guinea-pig colon

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Abstract—The possible role of opioid systems in the adaptive changes which follow chronic sympathetic denervation in the guinea-pig colon has been studied by comparing the effects of the opioid agonist morphine in control animals and after chronic sympathetic denervation. Supersensitivity to the inhibitory effects of morphine on the peristaltic reflex was observed after chronic sympathetic denervation, while the potency against acetylcholine release was unmodified. Our results suggest that a modification of the opioid system occurs after sympathetic denervation in the guinea-pig colon. Supersensitivity to endogenous opioids at a site different from that regulating acetylcholine release could account for the counter-regulation of intestinal motility after chronic sympathetic denervation.

The observation that α_2-adrenoceptor blockade by yohimbine causes an increase in propulsion velocity and acetylcholine (ACh) release in the guinea-pig colon suggests the existence of an adrenergic inhibitory tone through the activation of α_2-heteroreceptors (Marcoli et al 1985). Accordingly, sympathetic denervation should be expected to increase ACh release and the efficiency of the peristaltic reflex. In our experiments, however, no increase in either parameter was observed 6 days after sympathetic denervation (Frigo et al 1984; Marcoli et al 1985). The difference between the effects of acute and chronic suppression of the adrenergic inhibitory tone may be accounted for by the occurrence of adaptive changes after sympathetic denervation. Intrinsic non-adrenergic inhibitory systems able to control intestinal motility and cholinergic neurotransmission could be involved in such adaptive changes. Evidence, including the ability of the opioid antagonist naloxone to increase ACh release or integrated motility, suggests a physiological role for endogenous opioids in modulating intestinal intrinsic pathways (Kromer 1988). To study the possible involvement of opioid systems in the aforementioned adaptive changes, occurring after chronic sympathetic denervation, the effect of the opioid agonist morphine in inhibiting peristaltic reflex and ACh release has been investigated in control preparations and after chronic sympathetic denervation in guinea-pig colon.

Materials and methods

Experiments were carried out in isolated distal colon taken from male guinea-pigs (200–300 g). Potency of morphine in inhibiting peristaltic reflex and endogenous ACh release was measured in control animals and in experimental animals 6 days after sympathetic denervation. Sympathetic denervation was carried out surgically by freezing the periarterial plexus of the inferior mesenteric artery and by removing the inferior mesenteric ganglion as described by Frigo et al (1984). Sham operated animals were taken as controls.

The peristaltic reflex was elicited by a radial distension of the lumen applied at the proximal end of specimens 5–7 cm long by means of an intraluminal balloon. Longitudinal muscle movements and displacement of the balloon towards the distal end of the specimen were recorded. Since the velocity of propulsion is dependent on the degree of distension (Frigo & Lecchini 1970), supramaximal stimulation was employed and the maximal velocity of propulsion taken as a measure of the efficiency of the peristaltic index.

Resting ACh release was measured from specimens under 0·5 g tension in a 3 mL organ bath containing Tyrode solution with added physostigmine sulphate (15 μM) according to Frigo et al (1984). The incubation medium was collected every 20 min. ACh in the medium was assayed on the guinea-pig isolated ileum in the presence of morphine sulphate (6·6 μM) and physostigmine sulphate (7·7 nM) to increase the sensitivity of the bioassay according to the method of Paton & Vizi (1969). To allow for any effect that modifying drugs might have on the ACh assay, the concentrations of such drugs in the test samples were reproduced in the standard ACh solution during the assay.

The Tyrode solution was of the following composition (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·04, $NaHCO_3$ 11·9, NaH_2PO_4 0·4 and glucose 5·5.

The drugs used were: acetylcholine chloride (ACh, Sigma Chemicals, St Louis, MO, USA); morphine hydrochloride (SIFAC, Milan, Italy); (−)-naloxone hydrochloride (Sigma Chemicals); physostigmine sulphate (Sigma Chemicals).

Linear regression analysis of the log concentration response relationships was performed and the concentrations able to produce half-maximum effect (EC50) calculated according to Tallarida & Jacob (1979). Statistical significance of the differences between groups was analysed by applying Student's *t*-test for unpaired data.

Results

The efficiency of the peristaltic reflex and the rate of endogenous ACh release were not affected by chronic sympathetic denervation (6 days), propulsion velocity being (mean ± s.e.m., n = 12) 1·57 ± 0·23 and 1·44 ± 0·34 mm s^{-1} and ACh release (mean ± s.e.m., n = 9) 32·4 ± 2·9 and 28·5 ± 4·1 ng g^{-1} min^{-1} in control and in denervated organs, respectively.

Supersensitivity to morphine in inhibiting propulsion velocity was observed after chronic sympathetic denervation (6 days), the EC50 values (with 95% confidence limits) being 0·19 (0·10–0·34) μM in control and 0·034 (0·016–0·068) μM in denervated organs (Fig. 1a). Naloxone 0·03 μM increased by 20·1 ± 2·5% (mean ± s.e.m., n = 3) and 24·0 ± 3·1% (mean ± s.e.m., n = 3) the propulsion velocity in control and in sympathetically denervated organs, respectively. The potency of morphine in inhibiting ACh release did not differ after chronic sympathetic denervation with respect to the controls (Fig. 1b).

Discussion

In the present experiments, supersensitivity to the inhibitory effect of morphine on the peristaltic reflex in guinea-pig colon was observed after chronic sympathetic denervation. If sensitivity changes to endogenous transmitters can be inferred from changes in sensitivity to exogenously added receptor agonists, our results suggest that supersensitivity to endogenous opioids could develop after sympathetic denervation and account for the counter-regulation of intestinal motility. Changes of central and

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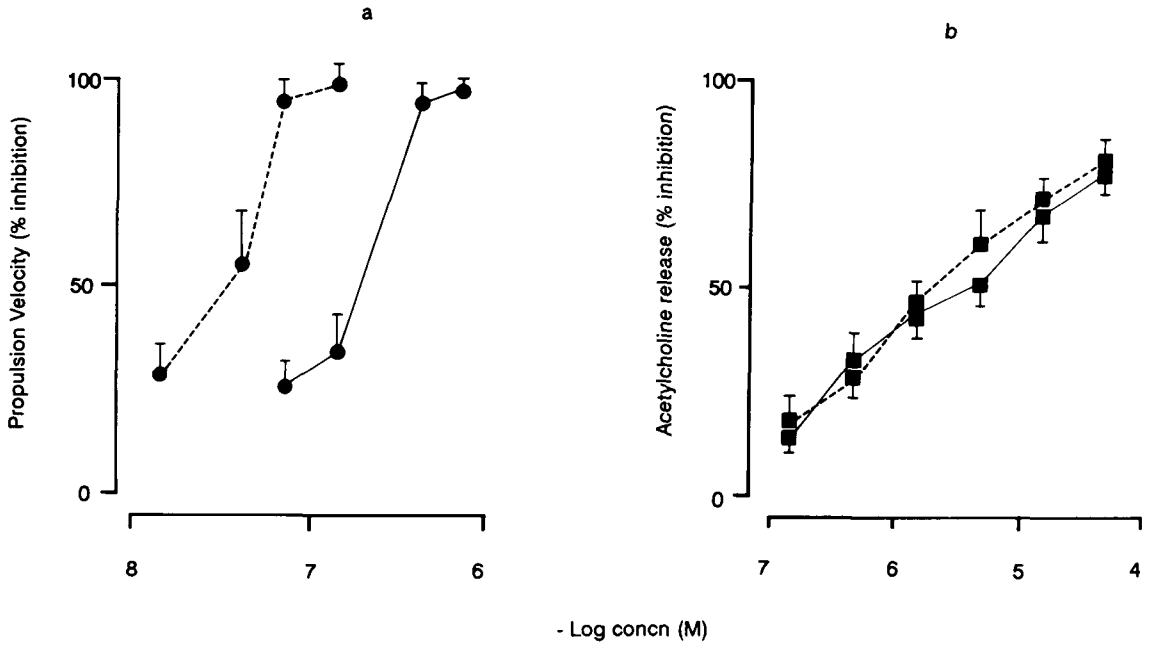

FIG. 1. Supersensitivity to morphine after chronic sympathetic denervation in guinea-pig colon. Log concentration-effect relationships for morphine in inhibiting (a) propulsion velocity (●) and (b) resting endogenous ACh release (■) in control preparations (——) and 6 days after sympathetic denervation (- - -) are shown. Drug effect was measured as percent inhibition of propulsion velocity or ACh release with respect to the control values. Each point represents the mean of 5 experiments. Vertical bars indicate s.e.m.

peripheral opioid receptors have been reported to follow chronic modification of the adrenergic transmission; down regulation of opioid receptor or subsensitivity to opioids was induced by tricyclic antidepressant treatment in central and peripheral nervous systems (Reisine & Soubrie 1982; Keith & Salama 1987). In the guinea-pig ileum, endogenous opioid level might be increased by adrenoceptor activation (Schulz et al 1986). If the endogenous opioid pathway was activated by adrenergic transmission also in the colon, then it would be expected to undergo modification following sympathetic denervation. In fact, supersensitivity to endogenous opioids could be related to an impairment of opioid transmission in sympathetically denervated organs. Further study is required to assess the above hypothesis. In any case, facilitation of peristaltic reflex by the opioid antagonist naloxone and supersensitivity to morphine in sympathetically denervated (6 days) organs are suggestive that modification of the endogenous opioid pathway following sympathetic denervation involves changes at postsynaptic receptors.

The lack of changes in morphine potency against ACh release and supersensitivity to morphine in inhibiting propulsion in the sympathetically denervated colon is consistent with different sites being responsible for the opioid regulation of peristaltic reflex and ACh release (Kromer & Schmidt 1982). It is highly suggestive that the opioid systems could play a relevant role in maintaining the inhibitory tone on the intestinal motor activity after sympathetic denervation. Changes of other intrinsic systems modulating ACh release could possibly account for the regulation of cholinergic neurotransmission after chronic sympathetic denervation.

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