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Effect of β-Casomorphins on Intestinal Propulsion in the Guinea-pig Colon

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Abstract—β-Casomorphins are a family of opioid peptides originally isolated from β-casein. In view of a possible physiological significance of these milk-derived compounds, the effects of bovine β-casomorphin-5 (β-CM-5), β-casomorphin-4 (β-CM-4) and D-Ala^2-β-casomorphin-4-NH$_2$ (D-Ala^2-β-CM-4-NH$_2$) have been investigated on the peristaltic reflex in the guinea-pig isolated colon and compared with morphine. β-CM-5 and D-Ala^2-β-CM-4-NH$_2$ each dose-dependently inhibited the velocity of propulsion of an intraluminal bolus; β-CM-4 was ineffective. IC50 values were 0·30, 5·21 and 0·29 μM for morphine, β-CM-5 and D-Ala^2-β-CM-4-NH$_2$, respectively. The potency ratios vs morphine were 0·06 and 0·96 for β-CM-5 and D-Ala^2-β-CM-4-NH$_2$, respectively. Blockade of the peristaltic reflex by β-CM-5 or D-Ala^2-β-CM-4-NH$_2$ was reversed by the opioid antagonist naloxone. D-Ala^2-β-CM-4-NH$_2$ also dose-dependently inhibited resting acetylcholine output (IC50 = 5·69 μM; potency ratio vs morphine: 0·63). In conclusion, certain β-casomorphins inhibit intestinal propulsion and cholinergic neurotransmission in the guinea-pig colon, probably by acting at opioid receptors.

Bovine β-casomorphins are opioid peptides originally isolated from the milk protein β-casein (Brantl & Teschemacher 1979; Brantl et al 1981). They are obtained by successive C-terminal amino acid cleavage of the 60-66 fragment of bovine β-casein. They each contain the common *N*-terminal amino acid sequence Tyr-Pro-Phe-Pro, differing from that of other endogenous opioid peptides (e.g. enkephalins, β-endorphin and dynorphin) and possess preferential μ-receptor agonist activity (Brantl et al 1981). The ability of β-casomorphins to interact with opioid receptors has been documented both in opioid receptor binding and bioassay studies (Brantl et al 1981; Chang et al 1982; Pasternak 1988). Among naturally-occurring β-casomorphins, β-casomorphin-5 was the most potent compound in several assays (Brantl et al 1981). β-Casomorphin-4 is less active, but its C-terminal amide derivative is a highly active and selective agonist for the μ opioid receptor (Chang et al 1981). Replacement of Pro^2 by D-Ala^2 in the β-casomorphin structure increases opioid activity and makes β-casomorphins less susceptible to degradation by proteolytic enzymes, such as dipeptidyl-peptidase IV (Brantl et al 1982; Kreil et al 1983).

β-Casomorphins are released in the human intestine after enzymatic degradation of bovine β-casein (Svedberg et al 1985). Oral or intragastric administration of β-casomorphins in dogs increases postprandial insulin, somatostatin and pancreatic polypeptide plasma levels (Schusdziarra et al 1983a, b, c).

In view of the possible physiological significance of β-casomorphins as modulators of intestinal function, we evaluated the effects of β-casomorphin-5, β-casomorphin-4 and D-Ala^2-β-casomorphin-4-NH$_2$ on the peristaltic reflex in the guinea-pig isolated colon and the effect of D-Ala^2-β-casomorphin-4-NH$_2$ on acetylcholine output from the myenteric plexus.

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Materials and Methods

Peristaltic reflex

Male guinea-pigs (300-400 g) were used. A 10 cm segment of the distal colon was removed and mounted in a 100 mL organ bath which was perfused with Tyrode solution at 35·5°C, continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode solution was as follows (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 peristaltic reflex was elicited by a localized intraluminal distension every 10 min by means of an intraluminal balloon (Crema et al 1970; Frigo & Lecchini 1970). The velocity of propulsion was measured by recording the displacement of the intraluminal bolus. Care was taken to achieve a degree of distension able to produce the maximal velocity of propulsion. The latter was considered as a measure of the efficiency of the peristaltic reflex. In order to assess the effect of β-casomorphins and morphine on the peristaltic reflex, the compounds were added to the bath 3 min before applying the distending stimulus. Drug-induced inhibition of propulsion was expressed as a percentage variation of the propulsion velocity and estimated as described by Frigo et al (1984). In some experiments, the ability of 10 min incubation with naloxone to remove the casomorphin-induced blockade of the peristaltic reflex was assessed.

Acetylcholine release

Acetylcholine release under resting conditions was evaluated using the standard procedure for biological assay (Paton & Vizi 1969; Lecchini et al 1969). A 2-3 cm long segment of the distal colon was mounted isotonically (weight 500 mg) in a 3 mL organ bath of Tyrode solution containing 15 μM physostigmine sulphate. Samples of the incubation medium were collected every 20 min and evaluated for acetylcholine concentration against suitable standards. Acetylcholine was assayed on the isolated guinea-pig ileum incubated with

morphine hydrochloride (6·6 μM) and physostigmine sulphate (7·7 nM). The sensitivity of the preparation allowed the detection of responses to acetylcholine at concentrations as low as 0·2 ng mL^{-1}. To allow for any effects that modifying drugs might have on the acetylcholine assay, the concentrations of such drugs in the test samples were duplicated in the standard acetylcholine solutions during the assay. Drug effects on acetylcholine output were expressed as percentage variations with respect to control levels.

Statistical analysis

Concentration-response relationships for each drug were calculated by linear regression analysis according to Finney (1978). The best linear fitting of the concentration-response curves was obtained by the log transformation and the least squares method. IC50 values (i.e. the concentration that reduced by 50% the velocity of propulsion or resting acetylcholine output) and potency ratios (with 95% confidence limits) were derived from log concentration-response relationships according to Finney (1978).

Drugs

The drugs used were: morphine hydrochloride (SIFAC, Milan); naloxone hydrochloride and physostigmine sulphate (Sigma). Bovine β-casomorphin-5 (β-CM-5), β-casomorphin-4 (β-CM-4) and D-Ala^2-β-casomorphin-4-NH_2 (D-Ala^2-β-CM-4-NH_2) were kindly donated by Dr. Victor Brantl (Boehringer Ingelheim). Drug concentrations are expressed on a molar basis.

Results

Peristaltic reflex

Experiments on the peristaltic reflex were conducted in 24 preparations. β-CM-5, D-Ala^2-β-CM-4-NH_2 and morphine dose-dependently inhibited the efficiency of the peristaltic reflex. Dose–response relationships are reported in Fig. 1. β-CM-4 had no effect on this parameter up to 20 μM.

IC50 values for inhibition of the peristaltic reflex (with 95% confidence limits) were 0·30 (0·11–0·80), 5·21 (2·32–11·67) and 0·29 (0·15–0·54) μM for morphine, β-CM-5 and D-Ala^2-β-CM-4-NH_2, respectively. The potency ratios vs morphine were 0·06 (0·04–0·08) and 0·96 (0·72–1·29) for β-CM-5 and D-Ala^2-β-CM-4-NH_2, respectively.

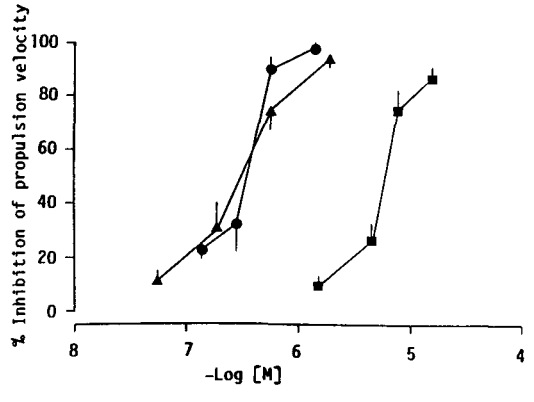

FIG. 1. Opioid-induced inhibition of propulsion velocity in the guinea-pig colon. Log concentration-response relationships for morphine (●), β-CM-5 (■) and D-Ala^2-β-CM-4-NH_2 (▲). Each point represents the mean ± s.e.m. from four experiments.

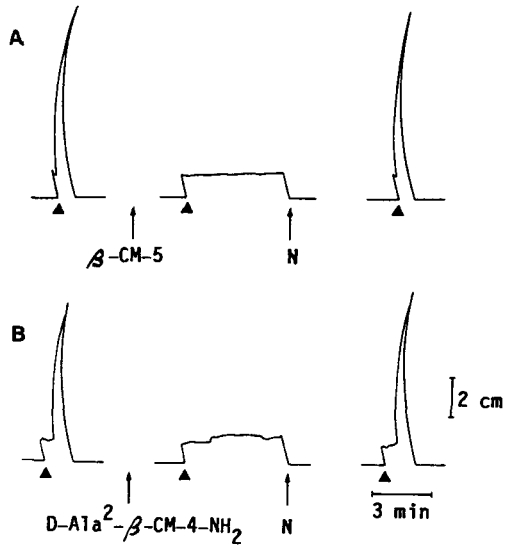

FIG. 2. Reversal by 15 nM naloxone (N) of blockade of the peristaltic reflex induced by 15 μM β-CM-5 (A) and 2 μM D-Ala^2-β-CM-4-NH_2 (B). Application of the distending stimulus at ▲.

The effect of naloxone was tested in six preparations. Blockade of the peristaltic reflex by β-CM-5(7·5–15 μM, n = 3) and D-Ala^2-β-CM-4-NH_2 (0·55–2 μM, n = 3) was reversed by 15 nM naloxone in all preparations (Fig. 2) tested.

Acetylcholine release

Sixteen preparations were investigated. Morphine and D-Ala^2-β-CM-4-NH_2 each inhibited resting acetylcholine output in a dose-dependent manner (Fig. 3).

IC50 values for inhibition of acetylcholine output (with 95% confidence limits) were 3·53 (1·44–8·67) and 5·69 (1·78–18·25) μM for morphine and D-Ala^2-β-CM-4-NH_2, respectively. The potency ratio of D-Ala^2-β-CM-4-NH_2 vs morphine was 0·63 (0·34–1·15).

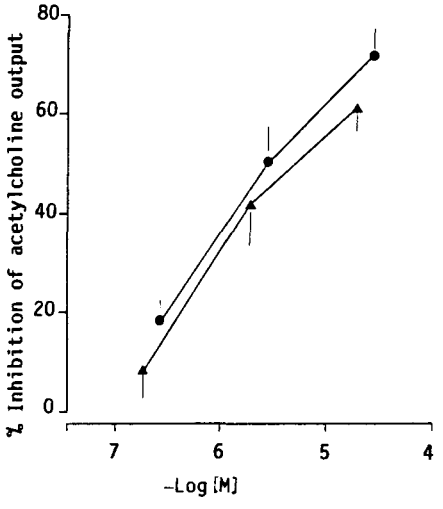

FIG. 3. Opioid-induced inhibition of resting acetylcholine output in the guinea-pig colon. Log concentration-response relationships for morphine (●) and D-Ala^2-β-CM-4-NH_2 (▲). Each point represents the mean ± s.e.m. from four experiments.

Discussion

The function of enteric cholinergic neurons can be modulated by activation of both autoreceptors and heteroreceptors. Indeed, it is well known that acetylcholine output from the guinea-pig myenteric plexus is inhibited by muscarinic, opioid and α_2-adrenoceptor agonists (Frigo et al 1984; Vizi et al 1984; Kilbinger & Nafziger 1985; Marcoli et al 1985). Moreover, several enteric neurotransmitters, such as 5-hydroxytryptamine, GABA and peptides, affect cholinergic neurotransmission. However, the physiological role of endogenous opioids is still under discussion (Furness & Costa 1982). β-Casomorphins represent a group of "exogenous" opioids which might be involved in the physiological modulation of enteric neurons. The present study has shown that: some β-casomorphins can inhibit the peristaltic reflex and acetylcholine output in the guinea-pig isolated colon; D-Ala^2-β-CM-4-NH_2 was approximately equipotent to morphine; β-casomorphins act as opioid agonists in the guinea-pig colon, since their effect in inhibiting the peristaltic reflex was reversed by naloxone.

The mechanism by which activation of opioid receptors by morphine and related opioid peptides suppresses intestinal motor function is only partially understood, although, at least in the guinea-pig, their neurogenic mechanism is well established (Furness & Costa 1982). In fact, one of the main mechanisms by which opioids inhibit intestinal motor activity is through an interference with neurotransmitter release at cholinergic synapses (Kosterlitz & Lees 1964; Vizi et al 1984). On the other hand, substances able to inhibit acetylcholine release by activation of different receptor mechanisms (e.g. opioids, α_2-adrenoceptor agonists) are well known and highly potent inhibitors of the peristaltic reflex (Frigo et al 1984; Donnerer & Lembeck 1985). Also, in the present experiments, the parallel inhibition of acetylcholine release and peristaltic reflex is highly suggestive of a role for cholinergic neurotransmission impairment in the inhibition of propulsive activity.

The question as to whether β-casomorphins play a physiological role and are able to mimic the well known effects of endogenous opioids is still open. In the enteric nervous system, both enkephalinergic and dynorphinergic neurons are widely distributed (Furness & Costa 1987) and endogenous opioid peptides affect a number of gastrointestinal functions, including motor activity, acid and endocrine secretions, intestinal fluid and electrolyte transport (McKay et al 1981; Konturek et al 1983; Jian et al 1987). β-Casomorphins can be considered as "exogenous" opioid peptides, which can be isolated from the β-casein of several species, including man (Brantl & Teschemacher 1979; Petrilli et al 1984; Koch et al 1985). However, for a physiological relevance of these food-derived peptides to be considered, the following conditions must be satisfied. First, β-casomorphins (or their precursors) must be released in the intestinal lumen by enzymatic digestion of milk casein. In-vivo studies in humans have indeed confirmed that β-casomorphins are released in the small intestine after ingestion of milk (Svedberg et al 1985). Second, β-casomorphins must be able to cross the mucosal barrier. So far, the extent to which the β-casomorphins are absorbed and therefore become systemically active is unknown, since they are degraded by enzymes of the intestinal mucosa (Caporale et al 1985). In-vitro studies (Hautefeuille et al 1986; Tome et al 1987) indicate, indirectly, that synthetic β-casomorphin analogues cross the epithelial barrier. β-Casomorphin immunoreactivity has also been detected in plasma of newborn calves after the first milk intake (Umbach et al 1985). This was not the case in adult volunteers (Teschemacher et al 1986), although this discrepancy might simply reflect differences in the mucosal barrier between newborns and adults.

In conclusion, measurement of the efficiency of the peristaltic reflex together with acetylcholine output is a suitable and sensitive method to detect any significant effects on enteric neuronal function. Provided a sufficient amount of active peptides is released during digestion, our results are compatible with some role of β-casomorphins in the control of intestinal motor responses through a modulation of local enteric reflexes.

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