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Modulation by $GABA_B$ and delta opioid receptors of neurally induced responses in isolated guinea-pig taenia coli and human colonic circular muscle

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Abstract — The GABA-ergic and opioid modulation of neurally induced muscle responses was studied in isolated guinea-pig taenia coli and human colonic circular muscle, using identical field stimulation parameters (rectangular pulses of 0.5 ms duration, 9 $V \cdot cm^{-1}$ intensity, trains of 3 pulses at 0.5 Hz, repeated every 1/3/5 min).The stimulation-induced contractions were inhibited in both preparations by GABA and baclofen; the IC_{50} values in human colonic circular muscle were ~100 and 31.0 μM, respectively. In guinea-pig taenia coli, the inhibition by 10^{-4} M GABA was dose-dependently reversed by 10^{-4}–10^{-3} M of $GABA_B$ receptor antagonist CGP 35348; antagonism by phaclofen was less effective in the same concentration range. In human colonic circular muscle, inhibition by 3×10^{-5} M baclofen was fully reversed by 10^{-3} M CGP 35348. With the exception of caecum, the delta 2 opioid receptor agonist deltorphin II was a potent inhibitor in human colonic circular muscle. 10^{-8} M Deltorphin caused a 74.4 ± 9.6% (n = 4) inhibition which was reversed by 10^{-6} M of delta receptor selective peptide antagonist BOC-Tyr-Pro-Gly-Phe-Leu-Thr(O^{t}Bu). Deltorphin II was ineffective in guinea-pig taenia coli even at 10^{-6} M; the same concentration caused an 84.3 ± 7.9 (n = 4) inhibition in human preparations. It is concluded that: 1) GABA-ergic modulatory mechanisms are present both in human colonic circular muscle and guinea-pig taenia coli; 2) the GABA receptors involved are of type B; and 3) delta opioid receptor-mediated modulation functions only in human colonic circular muscle in regions other than the caecum. © 2000 Elsevier Science Ltd. Published by Éditions scientifiques et médicales Elsevier SAS

guinea-pig taenia coli / human colonic circular muscle / $GABA_B$ receptors / delta opioid receptors

1. Introduction

Gamma aminobutyric acid (GABA) and proenkephalinA/prodynorphin-derived opioid peptides are neurotransmitters of enteric interneurons and comprise part of the intrinsic neural circuits regulating peristalsis [1, 2, 5, 6, 8]. Our study was aimed at characterising the receptor types where these transmitters exert their action in isolated, neurally stimulated human colonic circular muscle and guinea-pig taenia coli. The common technical motif for both preparations was the choice of parameters of field neural stimulation. These parameters (short trains at a low frequency) were expected to facilitate the detection both of inhibitory and stimulatory actions of exogenously administered receptor-specific agents. The chosen field stimulation parameters elicited a multiphasic or apparently monophasic muscle response with a dominant contractile component in both isolated organs ([9, 11] and Makó in preparation). The neural circuitry involved in the contractile responses of the two preparations is, naturally, quite different. The contractions were atropine-sensitive in guinea-pig taenia coli [11] whereas atropine-resistant in human colonic circular muscle ([9] and Makó in preparation). In the human colonic circular muscle, $5HT_2/H_1$ receptor mediated link is likely to be present in the stimulatory neural network since cyproheptadine inhibited the neural stimulation-induced contractions ([9] and Makó in preparation). To compare the opioid and GABA-ergic modulatory mechanisms in the two isolated organs, the delta (delta 2) opioid receptor agonist deltorphin II (DT-II), the delta receptor selective peptide antagonist BOC-Tyr-Pro-Gly-Phe-Leu-Thr(O^{t}Bu) [BOC-YPGFLT(O^{t}Bu)] [12], GABA, the $GABA_B$ receptor agonist baclofen and the $GABA_B$ receptor antagonist CGP 35348 and phaclofen [3, 4, 7, 10] were used as pharmacological agents.

2. Materials and methods

Drugs used were as follows: (-) baclofen (Research Biochemicals International), CGP 35348 (Tocris Neuramin), gamma-amino butyric acid (Sigma), phaclofen (Tocris Neuramin). BOC-Tyr-Pro-Gly-Phe-Leu-Thr(O^{t}Bu) was synthesised at the Research Group of Peptide Chemistry, Eötvös University, Budapest, as described previously [12]. Deltorphin II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2) was kindly supplied by G. Tóth of Isotope Laboratory, Biological Research Cen-

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tre of Hungarian Academy of Sciences, Szeged. All the other substances were of analytical grade.

Male guinea-pigs, weighing 250–350 g, were used and the taenia coli segments were prepared and mounted as described previously [11]. In brief, 5–8 mm long segments were mounted in Krebs' solution aerated with carbogen (O_2:CO_2 = 95:5) at 37 °C. An initial tension of 0.5 g was applied re-adjusted once after 5 min of 45–60 min equilibration period.

Subjects were 8 patients, 4 male and 4 female, age 28–76 years, undergoing colonic surgery for cancer (6) colitis ulcerosa (1) or slow colon (1). The preparations were made from non-diseased tissue dissected according to the pre-set rules of disease-dependent surgical protocols. Among the dissected tissues, the sigmoid colon (4), the rectum (4) and the caecum (1) were represented (i.e. from one subject, two specimens were dissected). The postoperative use of tissue samples was approved by the Ethical Committee of the University. The routinely used preoperative medication was metronidazole and aminoglycoside as antimicrobials and benzodiazepine as sedative. Five operations were performed under general anesthesia; in three cases, epidural anesthesia was used.

The specimens were transported in a special chamber in thermostated (33 °C) carbogenated Krebs' solution; the transportation took less than 20 min. The preparation was done at room temperature in carbogenated Krebs' solution. After the removal of the mucosal and serosal layer, 10–15 mm long, 2–2.5 mm wide circularly oriented strips were cut from the intertaenial segment. The strips were mounted in carbogenated Krebs' solution at 37 °C. The muscles were left without applying any tension for 15 min, washed once, then 1-g tension was applied. The tension was re-adjusted after 10 min then the preparations were equilibrated for 60 min under resting conditions. The composition of Krebs' solution was as follows (in $mmol \cdot L^{-1}$): NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2 (human) or 0.6 (guinea-pig), glucose 11.0.

The parameters of field stimulation [11] were: rectangular impulses of 0.5 ms duration, 9 $V \cdot c^{-1}m$ (supramaximal) intensity; trains of 3 pulses at 0.5 Hz frequency were repeated by 1 (guinea-pig) or 5 min (human). The contractions were measured under non-isometric conditions. From a single surgical specimen, 4–12 strips were prepared; they were randomly assigned to the different pharmacological interventions. In one preparation, only a single drug or drug combination was tested.

For statistical comparisons ANOVA followed by Student's *t*-test or just Student's *t*-test was used, depending on the experimental paradigms.

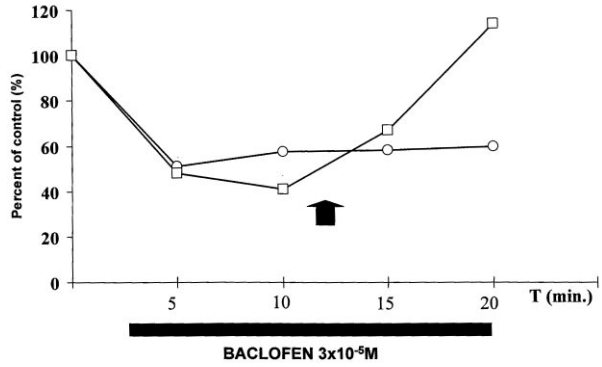

Figure 1. The inhibition of neural field stimulation-induced contractions of human colonic circular muscle by baclofen and the reversal of inhibition by CGP 35348. ○, 3×10^{-5} M baclofen alone; □, 3×10^{-5} M baclofen + 10^{-3} M CGP35348 added later (arrow). Points represent the mean of two independent experiments.

3. Results

Of the total of 65 human colonic circular muscle strips, 61 responded to neural field stimulation. Only those preparations were used for the pharmacological tests where an apparently monophasic contractile response was present with or without spontaneous background activity. Both GABA and baclofen inhibited the evoked contractions; in the case of the former, there was a high propensity to develop tachyphylaxis. At 10^{-4} M concentration, GABA exerted 55.4 ± 18.3% (mean ± SEM, $n = 3$) inhibition; at 10^{-3} M concentration, the inhibition tended to be less than 50%. Baclofen had a dose-dependent inhibitory effect with only a minor tendency for tachypylaxis; the IC_{50} value was 31.0 μM (28.6, 33.4, $n = 2$). The inhibition caused by 3×10^{-5} M baclofen could be reversed by the $GABA_B$ receptor antagonist CGP 35348 *(figure 1)*.

According to our previous findings in guinea-pig taenia coli [11], GABA (IC_{50} = 9.9 ± 3.8 μM, $n = 7$) and baclofen (IC_{50} = 4.5 ± 1.0 μM, $n = 5$) but not muscimol (at 10^{-4} M) had inhibitory effect in preparations stimulated neurally by parameters closely matching the ones used presently. We characterised further this inhibitory action by the reversal of the inhibition by 10^{-4} M GABA by cumulatively administered $GABA_B$ receptor antagonists CGP 35348 and phaclofen *(figure 2)*.

Besides GABA and baclofen, the delta 2 opioid receptor agonist deltorphin II was also a potent inhibitor in neurally stimulated human colonic circular muscle strips *(figure 3)* prepared from the sigmoid colon (3 patients) or rectum (2 patients) but not in preparations made from the caecum (1 patient).

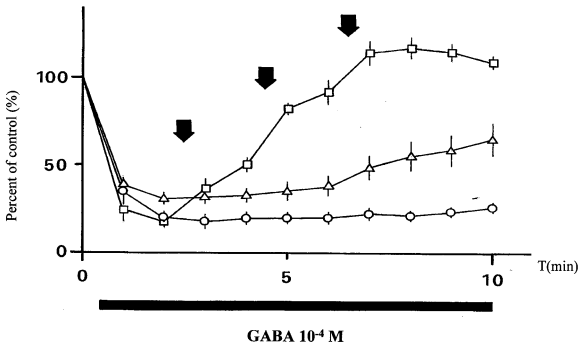

Figure 2. The inhibition of neural field stimulation-induced contractions of guinea-pig taenia coli by GABA and the reversal of inhibition by CGP 35348 or phaclofen. ○, 10^{-4} M GABA alone; □, 10^{-4} M GABA + 10^{-4}, 3×10^{-4} and 10^{-3} M CGP 35348 added in a cumulative manner (arrows); △, 10^{-4} M GABA + 10^{-4}, 3×10^{-4} and 10^{-3} M phaclofen. Points and vertical lines represent the mean ± SEM of 4 independent experiments. The reversal by CGP 35348 was significant at 10^{-4} M and onward (ANOVA and Student's *t*-test) whereas for phaclofen it became significant only at 10^{-3} M.

The washout was very slow and in most cases incomplete therefore in one preparation only a single concentration of DT-II could be tested. There was an 84.3 ± 7.9% (n = 4) inhibition at 10^{-6} M, 96.3 ± 3.0% (n = 3) at 10^{-7} M and 74.4 ± 9.6% (n = 4) at 10^{-8} M indicating that at these concentrations the effects fell into the 'plateau' phase of the dose-response curve and the IC_{50} must be below 10^{-8} M. The inhibition caused

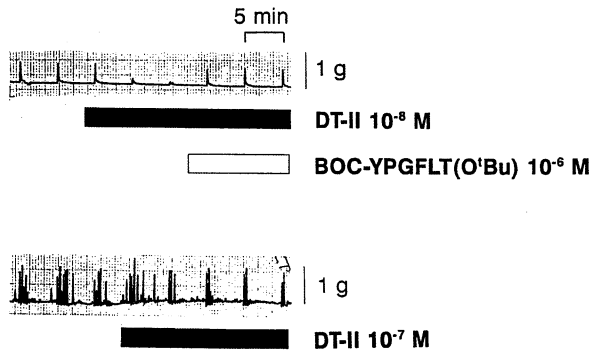

Figure 3. The inhibitory effect of deltorphin II on two types of neurally induced responses in human colonic circular muscle. Upper trace: inhibition of monophasic contractions by deltorphin II (DT-II) and the reversal of inhibition by BOC-Tyr-Pro-Gly-Phe-Leu-Thr(O^{t}Bu) (BOC-YPGFLT (O^{t}Bu). Lower trace: inhibition of polyphasic contractions.

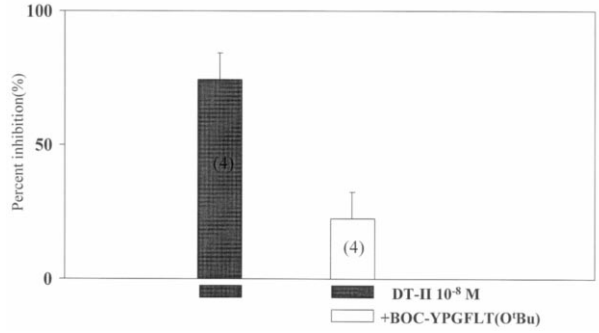

Figure 4. The reversal of the inhibitory action of deltorphin II by BOC-Tyr-Pro-Gly-Phe-Leu-Thr(O^{t}Bu) in neurally stimulated human colonic circular muscle. Bars and vertical lines represent the mean ± SEM of 4 independent experiments.

by 10^{-8} M DT-II could be readily reversed by the delta opioid receptor selective peptide antagonist BOC-YPGFLT(O^{t}Bu) *(figures 3, 4)*.

In circular muscle strips prepared from the caecum, DT-II did not affect neural stimulation-induced contractions even at 10^{-6} M (n = 3, not shown); in sigmoid colon-derived strips obtained from the same patient, DT-II was fully effective. Likewise, 10^{-6} M DT-II was ineffective in guinea-pig taenia coli (n = 4, not shown).

4. Discussion

Two types of inhibitory modulation was studied in two isolated organ preparations where the field-stimulation induced contractions have been shown to be either atropine-, (guinea-pig taenia coli, [11]) or tetrodotoxin sensitive (human colonic circular muscle, [9] and Makó in preparation) indicating the involvement of neural elements. It was shown presently that the GABA-ergic inhibitory modulation detected both in neurally stimulated human colonic circular muscle and guinea-pig taenia coli is mediated by B type of GABA receptors since 1.The action of GABA is mimicked by the $GABA_B$ receptor agonist baclofen; 2.the effect of either GABA or baclofen could be reversed by the $GABA_B$ receptor antagonist CGP 35348. Of the $GABA_B$ receptor antagonists we found CGP 35348 much more effective than phaclofen. In the CNS the localisation of CGP 35348 sensitive $GABA_B$ receptors has been characterised as preferentially „hetero-presynaptic» relative to GABA-ergic neurons [3, 10] i.e. they are present on the terminals of non-GABA-ergic neurons. The presence of GABA in mammalian enteric neural elements is extensively documented (e.g. [6]); in a subpopulation of human colonic neurons it has recently been de-

scribed to colocalise with enkephalin or nitric oxide synthase [8]. If the enteric localisation of CGP 35348 sensitive receptors follows the CNS pattern (i.e.they are hetero-presynaptic) we may assume either facilitatory $GABA_B$ receptors at nerve terminals releasing an inhibitory mediator, or inhibitory $GABA_B$ receptors at nerve terminals releasing an excitatory mediator.

Deltorphin II was a potent inhibitor of neurally mediated contractions in circular muscle strips from human sigmoid colon or rectum but not from caecum; likewise, it was ineffective in guinea-pig taenia coli. Delta and kappa opioid receptors are known to be present in human colon [1, 5]. Altough deltorphin II is a selective agonist of delta 2 subtype of opioid receptors in CNS tests [13], from the present results we cannot conclude that delta 2 receptors are present in human colonic circular muscle. First, hitherto we have been unable to detect delta 1/delta 2 opioid receptor heterogeneity in peripheral autonomic nerve/smooth muscle preparations (Rónai, unpublished) nor are we aware of such successful differentiation from other sources. Second, detailed further pharmacodynamic analysis is needed to make such a declaration for the human colonic circular muscle; at present the only valid statement is that these delta receptors are highly responsive to the agonist action of DT-II.

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