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Sodium butyrate and its synthetic amide derivative modulate nociceptive behaviors in mice

Roberto Russo[a], Carmen De Caro[a], Carmen Avagliano[a], Claudia Cristiano[a], Giovanna La Rana[a], Giuseppina Mattace Raso[a], Roberto Berni Canani[b], Rosaria Meli[a,*], Antonio Calignano[a]

[a] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy
[b] Department of Translational Medicine-Pediatric Section, University of Naples Federico II, Naples, Italy

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ABSTRACT

In the present study we investigated the role of sodium butyrate (butyrate), and its more palatable derivative, the *N*-(1-carbamoyl-2-phenyl-ethyl) butyramide (FBA), in animal models of acute and chronic pain. We found that oral administrations of butyrate (10–200 mg/Kg) or equimolecular FBA (21.2–424 mg/Kg) reduced visceral pain in a dose- and time-dependent manner. Both drugs were also effective in the formalin test, showing an antinociceptive effect. This analgesic effect was blocked by glibenclamide, suggesting the involvement of ATP-dependent K^+ channels. Moreover, following repeated administration butyrate (100–200 mg/Kg) and FBA (212–424 mg/Kg) retained their analgesic properties in a model of neuropathic pain, reducing mechanical and thermal hyperalgesia in the chronic constriction injury (CCI) model. The involvement of peroxisome proliferator-activated receptor (PPAR) -α and -γ for the analgesic effect of butyrate was also investigated by using PPAR-α null mice or the PPAR-γ antagonist GW9662. Western blot analysis, confirmed the role of peroxisome receptors in butyrate effects, evidencing the increase of PPAR-α and -γ expression, associated to the reduction of inflammatory markers (COX-2, iNOS, TNF-α and cFOS).

In conclusion, we describe the role of butyrate-based drugs in pain, identifying different and converging non-genomic and genomic mechanisms of action, which cooperate in nociception maintenance.

1. Introduction

Butyrate is a natural short-chain fatty acid (SCFA) which is present in dairy products and it is produced by commensal anaerobic fermentation of undigested carbohydrates in the colon [1]. Butyrate is the major energy source for colonocytes and it acts at intestinal level regulating epithelial cell proliferation, defense barrier, visceral sensitivity and intestinal motility [2]. Recent experimental evidence has suggested potential therapeutic applications for butyrate, including its utility in treating metabolic and inflammatory diseases [3,4].

The effects exerted by butyrate are multiple and different mechanisms of action have been proposed, including epigenetic modifications owing to its inhibitory effects on histone deacetylases (HDAC) [5], inhibition of NF-κB signaling [6], or direct agonism on the Free fatty acid receptor (FFAR)-2 and 3 (GPR41 and GPR43, respectively) [7]. Moreover, Alex et al. have also reported that the metabolic activity of butyrate is related to peroxisome proliferator-activated receptors (PPARs) [8], a class of nuclear receptors that serve a critical role in the control of inflammation and pain [9].

Clinical trials suggest that butyrate exerts its anti-inflammatory properties in human inflammatory bowel disease, including ulcerative colitis, proctosigmoiditis and chronic radiation proctitis [10–13]. Moreover, Luhrs et al. [6] showed that administration of butyrate to patients with ulcerative colitis suppressed mucosal inflammation, and decreased NF-kB activation in *lamina propria* macrophages. Recently, Vanhoutvin et al. showed that colonic

Abbreviations: SCFA, short-chain fatty acid; HDAC, histone deacetylases; GPR41 and GPR43 respectively, free fatty acid receptor (FFAR)-2 and 3; PPARs, peroxisome proliferator-activated receptors; MTC1, monocarboxylate transporter 1; CCI, chronic constriction injury; FBA, N-(1-carbamoyl-2-phenyl-ethyl) butyramide; IBS, irritable bowel syndrome; EGTA, ethyleneglycol-bis (β-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulphonylfluoride; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; ANGPTL4, angiopoietin-like protein 4.

* Corresponding author at: Via D. Montesano, 49; 80131 Naples, Italy. Fax: +39 081678403.
E-mail address: meli@unina.it (R. Meli).

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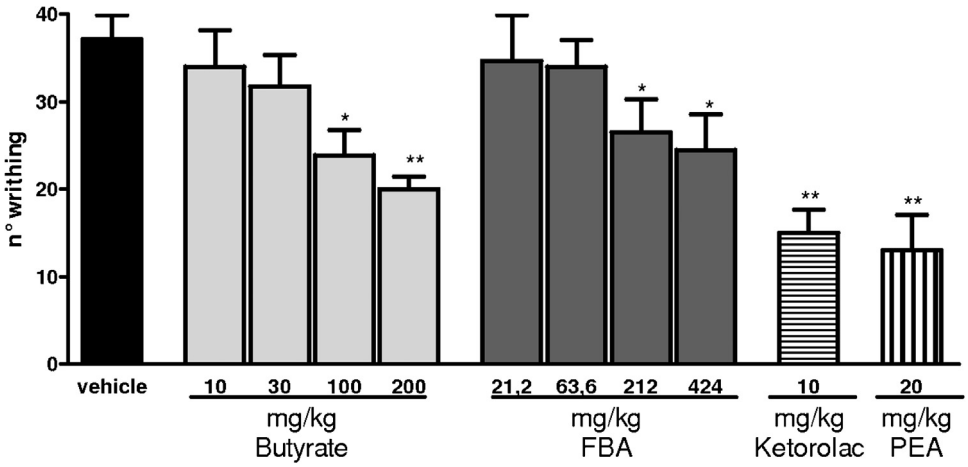

Fig. 1. Dose-dependent effect of Butyrate (10–200 mg/kg/os), phenylalanine-butyramide derivative (FBA; 21.2–424 mg/kg/os), ketorolac (Ketorolac, 10 mg/kg/os), and palmitoylethanolamide (PEA, 20 mg/kg/ip) on acetic acid-evoked writhing. Drugs were administered 1 h before acetic acid injection. Data are shown as mean ± SEM of 6 animals for vehicle, ketorolac and PEA, 8 animals for butyrate and FBA groups. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* vehicle group.

administration of butyrate decrease dose-dependently pain, urge, and discomfort in healthy human subjects [14].

On the other hand, preclinical studies on rats, showed that butyrate enemas prolong visceral hyperalgesia following trinitro-benzene sulfonic acid-induced colonic inflammation [15]. Furthermore, butyrate rectal instillation can be used as a model of non-inflammatory colonic hypersensitivity [16].

While local application of butyrate on distal colonic mucosa has been evaluated with discordant results [6,14–16], its beneficial effect after systemic administration on pain perception appears more clear, but little investigated. Recently, Kukkar et al. showed that oral administration of butyrate attenuates neuropathic pain symptoms in a chronic constriction injury (CCI) model [17], which may be mainly attributed to its ability to decrease the release of pro-inflammatory mediators during neuropathy development. Moreover, in irritable bowel syndrome (IBS) patients supplemental therapy with microencapsulated butyrate can reduce the frequency of selected clinical symptoms including abdominal pain [18].

Even if sodium butyrate is a more stable molecule and has a less rancid smell than butyric acid, its unpleasant taste and poor palatability represent the main limitations to the use of butyrate in humans.

In the present study we evaluated the dose- and time-dependent effects of butyrate in pain perception using different experimental models of acute and neuropathic pain. Furthermore, we investigated whether the analgesic properties of butyrate can be achieved using the *N*-(1-carbamoyl-2-phenyl-ethyl) butyramide (FBA), a more palatable butyrate-releasing compound [19].

2. Materials and methods

2.1. Animals

Male Swiss CD1 mice weighing 30–35 g were purchased from Harlan (Udine, Italy). They were housed in cages (6–8 mice for cages) in a room at 22 ± 1 °C on a 12/12 h light/dark cycle. Male wild-type (WT) and PPAR-α −/− (B6.129S4-SvJae-Pparatm1Gonz) mice previously backcrossed to C57BL6 mice for 10 generations, were bred in our animal house, where a colony was established and maintained by heterozygous crossing. Mice were genotyped as described on the supplier webpage (http://jaxmice.jax.org), with minor modifications. DNA was extracted from tails using the RedExtract kit (Sigma–Aldrich, Milan, Italy). All animals had free access to water and standard chow diet VRF1 (purchased from Special Diet Service—SDS-).

2.2. Ethics statement

Procedures involving animals and their care were conducted in conformity with international and national law and policies (EU Directive 2010/63/EU for animal experiments, ARRIVE guidelines and the Basel declaration including the 3R concept). The procedures reported here were approved by the Institutional Committee on the Ethics of Animal Experiments (CSV) of the University of Naples "Federico II" and by the Ministero della Salute under protocol no. 2014-0084607. At the end of all experiments for all pain models, the animals were euthanized by CO_2 overdose, none second method was used to verify euthanasia. As suggested by the animal welfare protocol, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.3. Chemicals and reagents

Sodium butyrate and all products used in this study were purchased by Sigma–Aldrich (Milano, Italy), palmitoylethanolamine (PEA) was purchased from Tocris (Tocris Bioscience Bristol, UK), phenylalanine-butyramide (FBA) was synthesized in our laboratories, as previously described (Italian patent RM2008A000214; April 21, 2008). Briefly, 0.01 M of phenylalanine carboximide and 0.01 M butyroyl chloride were dissolved in 50 ml of chloroform and the resulting mixture was left to react at room temperature for 24 h. The mixture, evaporated in vacuo, yields a solid white-color residue, that was washed with a 1% sodium bicarbonate solution. The aqueous bicarbonate solution was extracted twice with an equal volume of ethyl acetate to recover an additional fraction of the mixture of derivatives. To isolate the single components, the mixture was treated and processed chromatographically on a silica gel column, using dichloromethane as eluent. The compound was re-crystallised with a mixture of chloroform/n-hexane 1:1(v:v), obtaining a final yield equal to or greater than 50%. FBA is a solid, poorly hygroscopic, easily weighable form, stable to acids and capable of releasing butyric acid at small and large bowel level in a constant manner over time. This product has demonstrated a toxicological profile comparable to that of butyrate; it shows physicochemical characteristics distinctly more suitable for exten-