Involvement of the opioid system in the orexigenic and hedonic effects of melanin-concentrating hormone

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Lopez CA, Guesdon B, Baraboi ED, Monge Roffarello B, Hétu M, Richard D: Involvement of the opioid system in the orexigenic and hedonic effects of melanin-concentrating hormone. Am J Physiol Regul Integr Comp Physiol 301: R1105–R1111, 2011. First published July 20, 2011; doi:10.1152/ajpregu.00076.2011.—Melanin-concentrating hormone (MCH) exerts an orexigenic effect that resembles that of opioids, suggesting that the MCH and opioid systems could interact in controlling the food intake behavior. Three series of experiments were conducted in male Wistar rats: 1) to test the ability of the κ-, µ-, and δ-opioid receptor antagonists binaltorphimine (nor-BNI-κ), β-funaltrexamine (β-FNA-µ), and naltrindole (NTI-δ), respectively, to block the stimulating effects of MCH on food intake; 2) to verify the ability of MCH to induce a positive hedonic response to a sweet stimulus when injected into the nucleus accumbens shell (NACSh) or right lateral ventricle (LV) of the brain; and 3) to assess the ability of nor-BNI, β-FNA, and NTI to block the effects of MCH on the hedonic response to a sweet stimulus. Nor-BNI, NTI (0, 10 and 40 nmol), and β-FNA (0, 10 and 50 nmol) were administered into the LV prior to injecting MCH (2.0 nmol). To assess the hedonic response, rats were implanted with an intraoral cannula allowing for the infusion of a sweet solution into the oral cavity. Food intake was assessed in sated rats during the first 3 h following the MCH or vehicle (i.e., artificial cerebrospinal fluid) injection. The hedonic response to a sweet stimulus was assessed by examining facial mimics, following the intraoral administration of a sucrose solution. Blockade of each of the three opioid receptors by selective antagonists prevented MCH-induced feeding. Furthermore, MCH-injections into the NACSh and right LV resulted in enhanced hedonic responses. Finally, antagonism of the three opioid receptors blunted the LV-injected, MCH-induced, facial-liking expressions in response to an intraoral sweet stimulus. Overall, the present study provides evidence to link the MCH and opioid systems in the food intake behavior.

Further research showed that MCH has been described as an anabolic (promoting energy/fat gain) peptide (28, 31). It is prominently expressed in the lateral hypothalamus and zone incerta (5). Both chronic MCH treatment (15) and MCH overexpression (24) resulted in obesity and an increased susceptibility to high-fat feeding, whereas ablation of either MCH (39) or MCH neurons (1) promoted fat loss. In rodents, MCH produces its metabolic effects through a G protein-coupled receptor, the MCH receptor 1 (MCHR1) (31). Antagonism (38) and deletion of the MCHR1 (8, 27) led to leanness. There is clear evidence that the effects of MCH on energy balance are mediated through a stimulating action on energy intake and an inhibitory effect on energy expenditure (31).

The sites and mechanisms of the orexigenic action of the MCH are still uncertain. Recently, we demonstrated that bilateral injections of MCH into the nucleus accumbens shell (NACSh) caused an increase in food intake, which was comparable to that induced by the injection of MCH in the third ventricle of the brain, suggesting the importance of the NACSh as a site of the orexigenic action of MCH (19). The NACSh represents the brain area with the most noticeable expression of the MCHR1 (35). Since it is also rich in κ-, µ-, and δ-opioid receptors (25–26) and recognized as a prominent site of the orexigenic and hedonic response to opioids (16), one may hypothesize that there is a connection between the MCH and opioid systems in modulating the ingestive behavior.

The goal of the present study was to investigate the involvement of the opioid system in the effects of MCH on the ingestive behavior in rats. First, we tested the ability of the κ-, µ-, and δ-opioid receptor antagonists binaltorphimine (nor-BNI-κ), β-funaltrexamine (β-FNA-µ), and naltrindole (NTI-δ) to block the stimulating effects of MCH on food intake. Second, we verified the ability of MCH to enhance the hedonic response to a sweet stimulus, when injected into the NACSh and right lateral ventricle (LV). Third, we assessed the ability of nor-BNI, β-FNA, and NTI to block the stimulating effects of MCH on the hedonic response to a sweet stimulus. The hedonic response to a sweet stimulus was measured using the taste reactivity paradigm developed by Grill and Norgren (18).

ENERGY BALANCE REGULATION depends on complex controls exerted on both energy intake and energy expenditure. Those controls are essentially ensured by the hypothalamus, brainstem, and corticobulbar structures such as the insula, orbitofrontal cortex, and ventral striatum (4, 6, 12). All of the regions involved in energy balance produce chemical mediators capable of influencing food intake and energy expenditure (34), which include neuropeptide Y, proopiomelanocortin, endocannabinoids, opioids, and melanin-concentrating hormone (MCH).

MATERIALS AND METHODS

Animals and experiments. Rats (250–275 g) were housed individually in plastic cages and kept on a 12:12-h light-dark cycle with lights turned on at 07:00. They were fed a standard chow diet (cat. no. 5075 Rodent Laboratory Chow; Purina, Charles River, St-Constant, QC, Canada). Rats were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals. The protocols were approved by Université Laval’s Animal Care and Use Committee.
Three series of experiments were carried out in line with the objectives presented above. In the first series, we tested the ability of nor-BNI (κ-antagonist), β-FNA (μ-antagonist), and NTI (δ-antagonist) to block the stimulating effects of MCH on food intake by injecting the antagonists, followed by MCH, into the right LV of the brain. In the second series, we verified the ability of MCH to enhance the hedonic response to a sweet stimulus, when injected into the NAcSh or right LV. In the third series, we assessed the ability of nor-BNI, β-FNA, and NTI to block the stimulating effects of MCH on the hedonic response to a sweet stimulus (18). The antagonists and MCH were injected into the right LV during this third series.

**Surgery.** Rats (n = 140) were anesthetized with ketamine-xylazine (ketamine, 37.5 mg/ml; xylazine, 5 mg/ml) and implanted with the guide cannula (Plastics One, Roanoke, VA) above the right LV, using the following stereotaxic coordinates: 0.9 mm anterior to bregma, 1.2 mm lateral from midline, and 3.5 mm ventral to the brain surface (29). Additional rats (for the purpose of the second series of experiments only) were implanted with a guide cannula aimed at the NAcSh (n = 12), using the following stereotaxic coordinates: 1.7 mm anterior to bregma, 0.75 mm from midline, and 5.5 mm ventral to the brain surface (19). The cannula placement in the LV was confirmed by evaluating the dipsogenic response to angiotensin II (50 ng). The guide cannula was anchored to the skull with sterile stainless steel screws and acrylic cement. Stylets were inserted into the guide cannula to prevent occlusion. The cannula placement into the LV was further established by visual inspection of brain slices under a microscope at the end of the study. To facilitate that, rats were injected with methylene blue (into the LV) prior to death. The placement of the cannula into the NAcSh was histologically verified under a microscope on brains slices stained with thionin. The chronic intraoral cannula was secured as described by Grill and Norgren (18) and Cabanac and Lafrance (7). The cannula (heat-flared polyethylene PE-100 tubing) entered the mouth just lateral to the first maxillary molar, ascended laterally to the skull, and exited over the dorsal part of the skull, where it was attached with acrylic cement. This cannula allowed the direct infusion of solutions into the mouth for taste reactivity tests and did not interfere with the normal eating behavior (18).

**Drugs and microinjections.** Rat MCH (Sigma-Aldrich, Oakville, ON, Canada) was used at a dose of 2.0 nmol. β-FNA was used at doses of 10 and 50 nmol, whereas both nor-BNI and NTI were administered at doses of 10 and 40 nmol. The three antagonists were bought from Tocris Bioscience (Ellisville, MO). The doses of MCH and opioid antagonists were selected based on previous studies (21, 40). All drugs were dissolved in artificial cerebrospinal fluid (aCSF; Harvard Apparatus, Holliston, MA), which served as the vehicle.

Prior to every series of experiments, rats were handled and familiarized with the testing procedures for at least 3 days. Microinjections were carried out using a 50-μl Hamilton syringe connected to a syringe pump (4 μl/min for LV and 1 μl/min for NAcSh). While animals were gently handled, the injection cannula (30-gauge) was inserted into the guide cannula. The injection cannula projected into either the LV or the NAcSh. For each structure, the solution was carefully infused, for LV over 1 min and for NAcSh over 30 s. The antagonists or aCSF were first injected in the LV in a volume of 4 μl, followed by the injection of MCH (or aCSF) also in a volume of 4 μl. For the taste reactivity test in NAcSh, MCH (or aCSF) was injected bilaterally in a volume of 1 μl (0.5 μl per side) (19). In taste reactivity tests, each rat received tested drugs (MCH and opioid antagonists) or aCSF. The microinjections were performed every 4 days (for tests with opioid antagonists) or every 2 days (for tests with MCH alone). The choice of a 4-day interval was based on previous in vitro and in vivo studies, investigating the duration of the opioid receptor antagonist activity (19, 23).

**Food intake.** Food intake measurements were conducted in rats chronically implanted with a stainless-steel, 22-gauge guide cannula aimed at the right LV of the brain. Three distinct groups of animals were used to test each of the three opioid receptor antagonists. Each group received a unique dose of antagonist with injections of MCH or aCSF (4 μl).

Food intake was measured over 3 h between 11:00 and 15:00. Prior to the food intake measurements, rats were trained for 8 days to eat their daily ration of food (standard chow diet) between 17:00 and 08:00. This feeding schedule was designed to reduce appetite during the early light phase (41). Laboratory chow was used in feeding tests, which began 1 wk after surgery. The tests were performed as follows. All rats received a microinjection of the opioid receptor antagonist 90 min (for the κ- and δ-antagonists) or 22 h (for the μ-antagonist) prior to the MCH or aCSF injection. After the MCH or aCSF injections, which were performed between 10:30 and 11:50, rats were allowed to eat and drink water ad libitum. Food intake (in grams) was recorded at 1, 2, and 3 h following the MCH or aCSF injections. The time interval between the administration of the antagonist and MCH (or aCSF) was chosen based on previous investigations (2, 40).

**Hedonic response to a sweet stimulus.** The hedonic response to a sweet stimulus was assessed in rats implanted with an intraoral cannula, in addition to the guide cannula aimed at either the LV or NAcSh. Two distinct groups of rats were used to verify the ability of MCH (injected in the LV: n = 14 or NAcSh: n = 12) to induce a positive hedonic response to a sweet stimulus. Additionally, four groups of rats were used to further confirm the ability of MCH to induce a positive hedonic response to a sweet stimulus and to assess the ability of κ-, μ-, and δ-opioid receptor antagonists to block the stimulating effects of MCH on the hedonic responses.

MCH-induced hedonic response to a sweet stimuli was determined using the methodology developed by Grill and Norgren (18) for assessing the changes in palatability produced by neural or pharmacological manipulations. This technique has been extensively used by Berridge (3) and others (33).

The hedonic response tests began 2 wk after the surgery. To reduce the stress response during the test, animals were manipulated daily and habituated to the test chamber for 8 days and to the specific taste reactivity procedure for 4 days. The tests were performed by injecting the opioid receptor antagonists at a dose of 10 nmol prior to injecting MCH or the vehicle, as described above. The dose of 10 nmol for each antagonist was chosen based on our food intake results, and the order of MCH and vehicle administration was counterbalanced. Then 10 min after the MCH or aCSF injection, the oral cannula was connected to a polyethylene delivery tube (PE-50 tubing attached to a PE-10 nozzle), and the rat was placed in a Plexiglas test chamber. A mirror positioned beneath the transparent floor of the chamber reflected upon the face and mouth of the rat in the lens of the video camera. One milliliter of a 2% sucrose solution was infused manually into the mouth of the rat through the oral cannula by using a 1-ml syringe over 1 min. The hedonic reactions elicited by the sucrose taste were videotaped for subsequent analyses.

Hedonic reaction patterns were scored during a slow-motion (1/3 speed), double-blind, and frame-by-frame video analysis. Positive hedonic reactions included rhythmic midline tongue protrusions, lateral tongue movements, and paw licks (18). Neutral reactions, such as rhythmic mouth movements and passive drip of the solution were not scored. Aversive reactions such as gaps, headshakes, limb flails, and chin rubs were scored to probe that tested drugs did not alter the mobility of rats (data not shown). Separate occurrences were scored for each lateral tongue movement. Time bin scoring procedures were used to score behaviors that were produced in longer continuous bouts such as tongue protrusions (5 s bins) and paw licks (10 s bins). Use of these procedures allows multiple components to be combined into an overall hedonic reaction score (18). The double-blind video analyses were performed by two experienced individuals, and the scores of each individual were averaged.

The LV-cannulated rats subjected to the taste reactivity tests were also implanted with an intraoral cannula, and distinct groups of rats were used for each of the tested drugs (control, n = 9; 10...
nmol MCH + nor-BNI, n = 10; 10 nmol MCH + β-FNA, n = 14; 10 nmol MCH + NTI, n = 10).

Statistical analysis. Data obtained from rats with improper placement of cannula into the LV or NaClSh were excluded from the statistical analyses. Values are reported as means ± SE. In the first series of experiments two-way ANOVAs followed by Tukey-Kramer post hoc tests (when appropriate) were used to assess the differences between various conditions. In the second and third series of experiments two-way ANOVAs followed by Tukey-Kramer post hoc tests (when appropriate) were used to assess the differences between various conditions. In the second and third series of experiments two-way ANOVAs followed by Tukey-Kramer post hoc tests (when appropriate) were used to assess the differences between various conditions.

RESULTS

Food intake. Figures 1–3 illustrate the blocking effects of the κ-, μ-, and δ-opioid receptor antagonists on the MCH-induced feeding. The κ-opioid receptor antagonist nor-BNI inhibited the MCH-induced feeding at both 10 and 40 nmol doses during the 3 h of food intake measurements (Fig. 1). Nor-BNI at both 10 and 40 nmol doses also reduced food intake below that of rats not receiving MCH. At 1 h after injection, 10 nmol of nor-BNI reduced food intake by 39.1% compared with rats receiving double injections of vehicle i.e., aCSF/nor-BNI 0 (P < 0.05). In the presence of MCH, the 10 nmol dose of nor-BNI reduced food intake by 73.8% (MCH/nor-BNI 10 vs. MCH/nor-BNI 0; P < 0.0001). At the dose of 40 nmol, nor-BNI reduced the food intake by 83.4% in the absence of MCH (aCSF/nor-BNI 40 vs. aCSF/nor-BNI 0; P < 0.0001) and by 90.08% in the presence of MCH (MCH/nor-BNI 40 vs. MCH/nor-BNI 0; P < 0.0001). The significant (P < 0.05) effects of nor-BNI at the 10 and 40 nmol doses on food intake reduction were maintained over the 0 dose, for up to 3 h, in both MCH and aCSF-injected rats.

The μ-opioid receptor antagonist β-FNA also blocked the MCH-induced eating (Fig. 2). However, the blocking effect was achieved solely with the highest dose (50 nmol), and it persisted during the 3 h of measurement. In rats not receiving MCH, 10 nmol of β-FNA reduced food intake by 50.8% (aCSF/β-FNA 10 vs. aCSF/β-FNA 0; P < 0.05), whereas 50 nmol of β-FNA reduced food intake by 43.5% in the presence of MCH (MCH/β-FNA 10 vs. MCH/β-FNA 0; P < 0.0001).

The δ-opioid receptor antagonist NTI also blunted the orexigenic effect of MCH (Fig. 3). NTI at 10 nmol reduced food intake by 57.8% in the presence of MCH (MCH/NTI 10 vs. MCH/NTI 0; P < 0.05). In MCH-injected rats, the reducing effect of NTI at 10 nmol (over the 0 dose) lasted for 3 h. However, the effect of 40 nmol of NTI tended to disappear.

Fig. 1. Effect of the κ-opioid receptor antagonist nor-binaltorphimini (nor-BNI; 0, 10, and 40 nmol) on melanin-concentrating hormone (MCH)-induced eating. Three distinct groups of 6 rats were used to test each of the 3 antagonist doses (0, 10, and 40 nmol). Rats in each group received either MCH or artificial cerebrospinal fluid (aCSF). Nor-BNI or aCSF was injected 90 min prior to MCH or aCSF. The injections were made in the right lateral ventricle (LV). Food intake was measured at 1, 2, and 3 h in rats following the intracerebroventricular injection of MCH (2.0 nmol) or aCSF. Data are means ± SE. *P < 0.05, †††P < 0.0001, MCH vs. aCSF for every dose of nor-BNI, †P < 0.05, †††P < 0.0001, nor-BNI dose 10 or 40 vs. nor-BNI dose 0 on MCH and aCSF.

Fig. 2. Effect of the μ-opioid receptor antagonist β-funaltrexamine (β-FNA) (0, 10 and 50 nmol) on MCH-induced eating. Three distinct groups of 9 rats were used to test each of the 3 antagonist doses (0, 10, and 50 nmol). Rats in each group received either MCH or aCSF. β-FNA or aCSF was injected 22 h prior to MCH or aCSF. The injections were made in the right LV. Food intake was measured at 1, 2, and 3 h in rats following the intracerebroventricular injection of MCH (2.0 nmol) or aCSF. Data are means ± SE. *P < 0.05, **P < 0.001, ***P < 0.0001, MCH vs. aCSF, for every dose of β-FNA: †P < 0.05, ††P < 0.001, †††P < 0.0001, β-FNA dose 10 or 50 vs. β-FNA, dose 0 on MCH and aCSF.
after the 3 h of measurements (MCH/NTI 40 vs. MCH/NTI 0; *P > 0.5). We did not observe any anorexigenic effect of the δ-antagonist per se in the rats injected with aCSF instead of MCH.

**Hedonic response to an intraoral sweet stimulus.** Microinjections of MCH into either the NAcSh or right LV increased the total number of positive hedonic liking reactions elicited by the oral infusion of a sucrose solution, compared with the aCSF microinjections (Fig. 4). The number of rhythmic-tongue movements was increased by 50.2% and 64.38%, following the microinjections of MCH into the NAcSh and right LV, respectively. Similarly, the number of paw-licking movements was increased by 151% and 66.35% following the MCH microinjections in the NAcSh and right LV, respectively. No significant effect on the number of lateral tongue movements was observed, regardless of whether MCH was injected into the NAcSh or right LV.

Figure 5 illustrates the blocking effects of the κ-, μ-, and δ-opioid receptor antagonists on the MCH-induced hedonic responses. The injection of MCH into the right LV increased the total facial reactions by 67% compared with the vehicle (P < 0.05) (Fig. 5A). This result is consistent with the observations made in Fig. 4, confirming that MCH microinjection in the LV increased positive hedonic liking reactions. We retested the MCH effects in the right LV in context of the third series of experiments, where the animals were subjected to two intracerebroventricular injections, instead of one injection, made during the second series of experiments. As seen in Fig. 5, B, C, and D, the positive hedonic effects of MCH were no longer detectable following κ-, μ-, and δ-opioid receptor antagonism.

**DISCUSSION**

The present study demonstrated that the orexigenic effects of MCH can be blocked by the κ-, μ-, and δ-opioid receptor antagonists. Nor-BNI (κ-antagonist) induced a dose-dependent reduction in the orexigenic effect of MCH, which was apparently slightly stronger than that caused by β-FNA (μ-antagonist). NTI (δ-antagonist), in contrast to nor-BNI and β-FNA, showed its blocking effect primarily at the dose of 10 nmol. The present study further revealed that MCH injections into either the NAcSh or the right LV of the brain, could enhance the hedonic responses to an intraorally administered sweet
stimulus, which were blocked by each of the three opioid receptor antagonists.

The present findings are in line with the previous studies, which have shown that both the opioid and MCH systems are orexigenic (17, 28, 31) and have the ability to increase the food intake when injected into the NAc (19, 21). Also, both opioid (30) and MCH agonism (this study) can elicit a positive hedonic response when injected into the NAcSh. The NAcSh is known to exhibit the highest density of MCHR1 in the brain (36) and expresses \( \kappa \), \( \mu \), and \( \delta \)-opioid receptors (25–26) as well as neuronal projections releasing enkephalin (\( \delta > \mu \)), \( \beta \)-endorphin (\( \mu = \delta \)), and dynorphins (\( \kappa > \mu, \delta \)). Moreover, MCHR1 has been found in both the enkephalin- and dynorphin-positive spiny neurons of the NAcSh (14), suggesting that MCH could exert widespread effects on the opioid system. It is noteworthy that two studies have previously failed to block the orexigenic effect of MCH by antagonizing the opioid receptors (9, 13). However, in these studies, a nonselective opioid antagonist, naloxone, was used, which was administered intraperitoneally at a single dose.

The site of the MCH-opioid interaction on the feeding behavior has yet to be determined, and one of the limitations of the present work is that opioid antagonists were not injected into the NAcSh. Nonetheless, the NAcSh appears to be a strong candidate as the site for the MCH-opioid interaction. This nucleus is not only rich in opioid and MCH receptors, but is also an apparent site for both the MCHR1- and \( \mu \)-opioid receptor-mediated hedonic actions. Moreover, MCH microinjection in the NAcSh caused an increase in food intake that was equivalent to the increase caused by the LV injection of MCH (19), supporting the role of NAcSh as a key site for mediating the effects of MCH on the ingestive behavior. Further supporting the role for NAcSh in the orexigenic actions of MCH is the recent study by Sears et al. (37), which indicated that MCHR1-mediated effects on food intake occurred through a reduction in medium spiny neurons excitability in the NAcSh. Thus, the possibility of a MCH action into the NAcSh associated with an extra NAc opioid receptor-mediated effect cannot be excluded.

The mechanisms and pathways whereby the opioid antagonists block the orexigenic effects of MCH remain to be eluci-

![Fig. 5. Hedonic response to a sweet stimulus after an injection of MCH (2.0 nmol) or aCSF, in the absence (A; \( n = 9 \)) or in the presence of specific opioids receptors antagonist nor-BNI (B; \( n = 10 \)), \( \beta \)-FNA (C; \( n = 14 \)), and NTI (D; \( n = 10 \)). All of the injections were made in the right LV, the antagonists being given first. *\( P < 0.05 \).]
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dated. Since the majority of the enkephalin and dynorphin spiny neurons in the NAcSh express MCHR1 (14), one can conceivably argue that the MCH agonism in the NAcSh can implicate a coordinated action of those neurons in MCH orexigenic action. Enkephalin and dynorphin are ligands for the δ- and the κ-opioid receptors, respectively (20, 32), and can also block the μ-receptors at higher doses. Given that the blockade of each of the opioid receptors can block the orexigenic effect of MCH, one can argue that the opioid neurons act in a hierarchical manner. The respective and interactive roles of the δ- and κ-opioid receptors in the control of food intake have been less investigated than that of the μ-receptor (10–11, 42–43), but likely deserve attention in the light of the present results. Injection of nor-BNI was effective in reducing NPY-induced feeding, whereas the intraventricular injection of NTI did not decrease NPY-induced feeding at any dose. In addition, NTI, failed to alter nocturnal feeding and the feeding induced by deprivation, glucoprivation, or palatability (22).

In conclusion, the present study demonstrates that the orexigenic and hedonic effects of MCH can be blocked by κ-, μ-, and δ-opioid receptor antagonists, which suggests that the opioid and MCH systems interact in the control of the food intake behavior.

Perspectives and Significance

The present study was ultimately conducted to improve our knowledge on the brain pathways and circuits involved in energy balance regulation. Energy balance regulation depends on the controls exerted on both energy intake and energy expenditure. These controls are insured by different parts of the brain, including the hypothalamus, which contributes to the reflexive regulation of energy balance by integrating peripheral signals informing the brain about the fat stores and the nutritional status. The hypothalamus autonomically governs brown adipose tissue thermogenesis and controls food intake in close association with corticolimbic structures involved in rewards, learning, and motivation, constituting the reward (hedonic) system. The present study was carried out to further elucidate the pathways linking the hypothalamus to the hedonic parts of the brain. The emphasis was put on the interaction potentially existing between the MCH and opioid systems. MCH is synthesized in lateral hypothalamic neurons that project to the NAc where the MCHR1s are found abundantly and where opioid receptors mediate hedonic responses. By demonstrating that the MCH and the opioid systems interact in the control of food intake through influencing the hedonic responses to food, our study aids in deciphering the complex pathways/circuits underlying the ingestive behavior. Our findings open the door to further investigations to better understand the role of both the MCH and opioid systems in the regulation of energy balance.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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