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Summary: Negative expectations about headache lead to the enhancement of the cyclooxygenase-prostaglandins pathway, with consequent prostaglandins-related pain worsening, and placebos reduce this prostaglandins increase.

ABSTRACT

Nocebo and placebo effects have been found to modulate several neurochemical systems, such as cholecystokinin, endogenous opioids, and endocannabinoids. Here we show that also the cyclooxygenase-prostaglandins pathway can be modulated by both nocebos and placebos. In fact, we found that negative expectation, the crucial element of the nocebo effect, about headache pain led to the enhancement of the cyclooxygenase-prostaglandins pathway, which, in turn, induced pain worsening. As an experimental model, we studied hypobaric hypoxia headache at high altitude in 2 populations of subjects. Whereas the experimental nocebo group received negative information by a single individual who was informed about the risk of headache, the control group did not know about the possible occurrence of headache. We found a significant increase in headache and salivary prostaglandins and thromboxane in the nocebo group compared to the control group, suggesting that negative expectations enhance cyclooxygenase activity. In addition, placebo administration to headache sufferers at high altitude inhibited the nocebo-related component of pain and prostaglandins synthesis, which indicates that the cyclooxygenase pathway can be modulated by both nocebos and placebos. Our results show for the first time how nocebos and placebos affect the synthesis of prostaglandins, which represent an important target of analgesic drugs, thus emphasizing once again the notion that placebos and drugs may use common biochemical pathways.

Keywords: Nocebo, Placebo, Headache, Cyclooxygenase, Prostaglandins, Thromboxane, Cortisol.

1. Introduction

The nocebo effect is the negative counterpart of the placebo effect. Negative expectations of clinical worsening are crucial, so that the term nocebo effect is often replaced with negative expectation effect [2,6]. This holds true for the placebo effect as well, whereby the term placebo effect is frequently replaced with positive expectation effect. Several neurotransmitters have been identified in placebo and nocebo effects, such as the endogenous opioids, endocannabinoids, cholecystokinin [4,5,34]. Both placebo and nocebo effects can also be elicited by the mere observation of others [14,30,33], thus emphasizing that pain and analgesia can spread across individuals through complex cognitive mechanisms [3].

To examine the effects of negative expectations in a population of subjects, we studied hypobaric hypoxia headache, a typical symptom of acute mountain sickness, which is triggered by the drop in atmospheric oxygen pressure at high altitude [20,35]. One important factor triggering high altitude headache is represented by the acute effects of hypoxia on prostaglandins (PG) synthesis through the cyclooxygenase (COX) enzyme, with the formation first of PGH_2, and then of PGF_2, PGD_2, PGE_2, PGI_2 (prostacyclin), and TXA_2 (thromboxane A2) [27]. One of the most important effects of these eicosanoids is represented by vasodilation, which is thought to be the principal factor inducing acute hypoxia headache [8,15,19,23,26], although the direct stimulation of nociceptive afferents may also occur [21].

Patients suffering from migraine show PG and TX increases not only in plasma but in saliva as well, as reported in a number of studies, for example, in common migraine and menstrual migraine [16,24,25,31,32]. In addition, experimental and clinical evidence indicates that the blockade of PG synthesis with aspirin can prevent high altitude headache [7]. Therefore, on the basis of these considerations, we measured salivary PG and TX at high altitude, as already done for plasma PG [25].

We investigated the nocebo/placebo modulation of these eicosanoids by using at least 2 novel approaches. First, we investigated a clinical condition that is easily reproduced at high altitude, whereby the hypobaric hypoxic environment triggers headache as well as the activation of the COX-PG pathway. This makes high altitude headache an excellent and new model to investigate the effects of positive and negative expectations on this biochemical pathway. Second, we used a model of interindividual communication, whereby a single subject was informed about the risk of headache at high altitude. This subject (the "trigger") communicated the negative information to a subset of individuals, thus inducing negative expectations. Another subset of individuals was not informed about the risk of headache, and they represented the control group. Thus, in this experimental model, negative information was not communicated by the experimenter, but by the subjects themselves. By integrating the pathophysiological model of hypobaric hypoxia headache and the experimental model of interindividual negative communication, we analyzed the modulation of the COX-PG pathway by negative expectations. Then, the modulation by placebos was also investigated.

2. Material and methods

2.1. Procedure to induce negative expectations

A total of 121 students were invited to visit a research facility at an altitude of 3500m. Interaction among these students was guaranteed by their mutual contacts at the Medical and Nursing Schools of the University of Turin and Aoste, for they were enrolled in the same courses. All of them had no

experience with the high altitude environment, and no specific information was provided about possible headache occurrence. Negative expectations were not induced by the experimenters, but by the subjects themselves. To do this, we randomly chose a single subject (the "trigger") and informed him on the possible occurrence of severe headache (Fig. 1a). A flyer and a movie with a headache sufferer at 3500m lying on a bed, grimacing and taking pills, was presented to him. The "trigger" subject was also told that high altitude headache is very sensitive to aspirin, thus bringing aspirin to 3500m was highly recommended. In addition, he was told to contact us 2days before the trip to 3500 m in order to be sure about the doses of aspirin to bring with him. During the following week, we were contacted by 36 subjects asking for more details about high altitude headache and the doses of aspirin needed. These 36 students represented the subjects with negative expectations (nocebo group). In order to create the diagram of Fig. 1a, we interviewed them and asked from which student they had received information. The number of contacts for each individual were recorded, and they varied from 1 to 4 (Fig. 1a). To create a control group from the remaining 85 subjects, we adopted the following procedure. First we selected the number of individuals to be included in the control group, within the range 36 (subjects with negative expectations) ±5, and the number 38 was pulled out. Then we randomly extracted 38 subjects out of the remaining 85 subjects not reached by negative information.

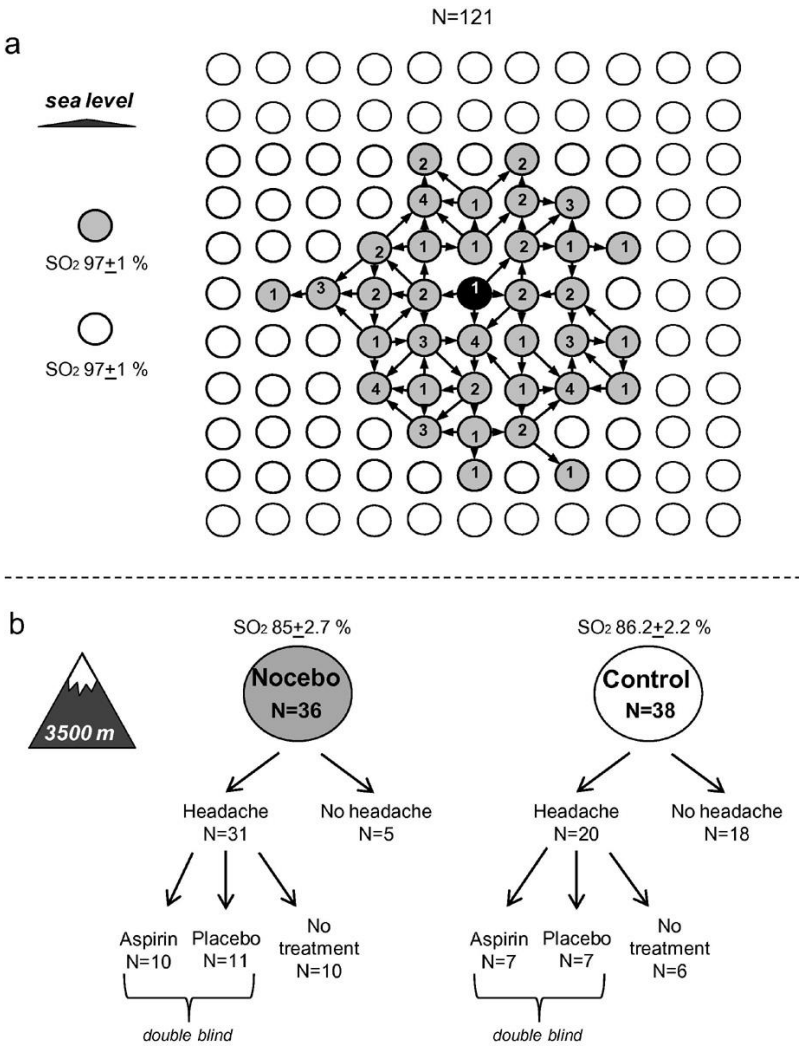

Fig. 1. Experimental design. (a) At sea level, 121 subjects were invited to go up to 3500 m. Only one of these (the ''trigger'': black circle) was informed about the risk of severe headache. The diagram shows the pattern of interindividual

negative communication that occurred naturally over a period of 1 week, in which 36 subjects received negative information (grey circles). The numbers represent how many times each individual received (inward arrows) the negative information. Mean (±SD) blood oxygen saturation (SO_2) is shown for those subjects who received negative information and those who did not. (b) At 3500 m, the nocebo group was represented by those subjects who received negative information, whereas the control group was represented by a subset of subjects (randomly chosen) who were not reached by the negative communication, thus, they did not receive any information about headache. Both groups were subdivided into headache and no-headache sufferers. Those with headache were further subdivided into those who took either aspirin or placebo and those who did not undergo any treatment. The drop in SO_2 in both groups shows the hypoxic condition.

None of these 38 students knew about the possible occurrence of headache and the use of aspirin, as assessed through a detailed interview. During the interview, all the subjects declared that they had never had experience with high altitude and that the only thing they expected was the possible shortness of breath. As a confirmation, at the end of the experiment, all the subjects were asked whether they had never suspected the possible occurrence of headache, and all of them answered "no." It is also worth noting that none of these subjects brought aspirin or any other analgesic with them to the high altitude laboratory.

2.2. Subjects and study location

The nocebo group (n=36) was represented by 20 females and 16 males (mean age 22±1.4years), whereas the control group (n=38) was represented by 24 females and 14 males (mean age 22.4±1.1years). The subjects reached 3500m from sea level in about 3 hours. All the experiments were performed during the first 20hours after reaching 3500m, thus, under acute hypobaric hypoxia. All subjects reached 3500m at about 5:00pm, and the experiment started at about 8:00 am and ended at about 1:00pm of the following day, thus guaranteeing that the measurements were performed at the same times of the day. The experiments were performed at the Plateau Rosa Research Station in the Matterhorn area at the Italian-Swiss border, at an altitude of 3500m, which was reached through 3 cableways. Here, air pressure is 490mm Hg (760mm Hg at sea level) and oxygen pressure is 102mm Hg (159mm Hg at sea level). This corresponds to a blood oxygen saturation in the range of 84%–90% (97%–98% at sea level), depending on different individuals. Ambient temperature inside the Research Station was always maintained at a comfortable range (20–22°C). All subjects signed a written informed consent, according to the rules and approval of our Institutional Ethics Review Board. In particular, the ethical approval included withholding information on high altitude-induced headache. This was deemed to be ethically acceptable, since most people going to high altitude are often unaware of some possible effects, such as insomnia, lack of appetite, nausea, fatigue, and headache. A medical examination, including electrocardiogram and spirometry, was carried out to rule out important pathologies.

2.3. Experimental protocol at 3500 m

In both the nocebo and control group, a first saliva sample was taken at sea level at 8:00 am on the same day of departure for 3500m. Then the subjects reached the high altitude location at about 5:00pm. The second sample was taken 15 hours after reaching 3500m, that is, at 8:00 am of the following day. Subjects were subdivided into those who reported headache and those who did not experience any headache pain (Fig. 1b). In addition to a numerical rating scale, ranging from 0=no pain to 10=unbearable pain, we also used the Lake Louis Scores for the assessment of acute mountain sickness. Then, the headache sufferers were randomly assigned to one of the following treatment groups: 1) aspirin at a dose of 25mg/kg, 2) placebo, 3) no treatment. The aspirin and the placebo group were studied according to a double-blind design. To do this, aspirin was dissolved in 150mL

of strawberry milk, whereas the placebo was represented by the strawberry milk alone. After 4 hours, at about 1:00pm, the third saliva sample was taken in the 3 groups (aspirin, placebo, no treatment) and pain assessed according to a numerical rating scale ranging from 0=no pain to 10=unbearable pain.

2.4. Salivary prostaglandins (PG) and thromboxane (TX)

Saliva samples were collected after stimulation with sterile 2% citric acid applied to the tip and sides of the tongue with a cotton-tipped applicator. To prevent mixing of stimulated and unstimulated saliva, the first 2 minutes of saliva was discarded. Then citric acid was continually applied for an additional 2 minutes or until 2mL of saliva was collected. Saliva was collected by means of a syringe. All samples were kept at -20°C until preparation for analysis. PG analysis started by thawing the saliva samples at room temperature and recording the volume of each sample. Then, the samples were centrifuged at 3000rpm at 4°C and the supernatant utilized for PG and total protein analysis. We determined all the main products of cyclooxygenase (Fig. 2), the enzyme that transforms arachidonic acid into PGH_2, which, in turn, is transformed into PGD_2, PGE_2, PGF_2, PGI_2, TXA_2. By using enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI, USA), we analyzed PGD_2, PGE_2, and 8-isoprostane PGF_{2a} (PGF_2) directly, whereas PGI_2 (prostacyclin) was analyzed by assessing its stable metabolite 6-keto PGF_{1a}, and TXA_2 assessed through its stable metabolite TXB_2 (Fig. 2). In order to control for artifact variance in the ELISA assay, the amount of PG was normalized to the volume of saliva collected and amount of total protein, which was determined using a standard protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

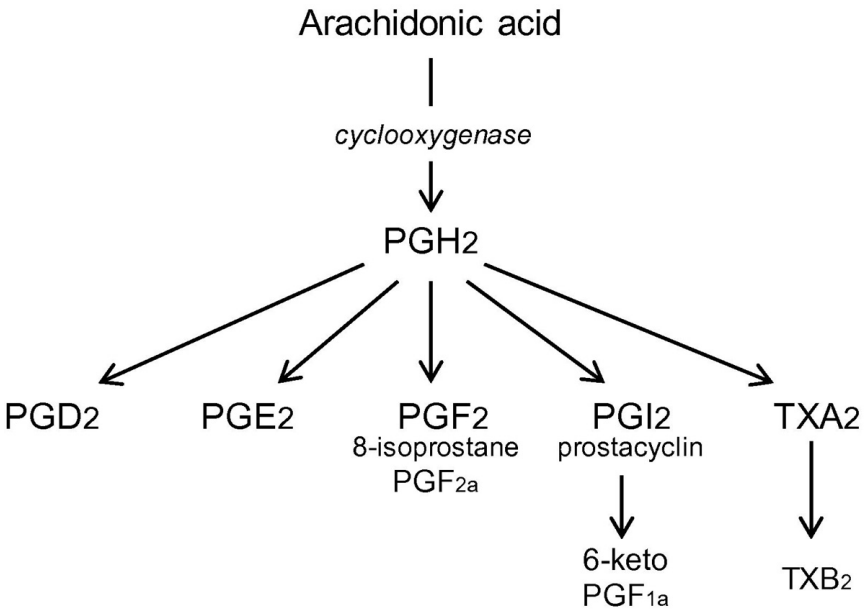

Fig. 2. Synthesis of prostaglandins (PG) and thromboxane (TX) from arachidonic acid through the enzyme cyclooxygenase.

2.5. Salivary cortisol

Cortisol analysis started by thawing the saliva samples at room temperature and by recording the volume of each sample. Then samples were centrifuged at 3000rpm at 4°C. A salivary Cortisol ELISA Kit (Marburg, Germany) was used to measure salivary cortisol concentrations. The range of the assay was 0.537–80ng/mL, and the intra- and interassay variability coefficients were 1.5–4.5% and 5.8–7.5%, respectively.

2.6. Additional group at 1500 m in nonhypoxic (normoxic) conditions

An additional group of 92 subjects underwent exactly the same procedures described above. The only difference was that they went up to 1500m, where no hypoxia is present. Here, air pressure is 648mm Hg and oxygen pressure 136mm Hg, which guarantees a blood oxygen saturation of 97%–98%, as at sea level. This group allowed us to establish whether the nocebo effect occurred even without hypoxic conditions. There were 24 subjects in this nocebo group (15 females, 9 males, mean age 21.8±1.2years), whereas 20 subjects (12 females, 8 males, mean age 22.1±1years) randomly taken from those not reached by negative information were used as a control. The same randomization procedure described above was used. First, we selected the number of individuals to be included in the control group, within the range 24 (subjects with negative expectations) ±5, and the number 20 was pulled out. Then we randomly extracted 20 subjects out of the remaining 68 subjects not reached by negative information.

2.7. Statistical analysis

Results are expressed as means and 95% confidence intervals (CI). Headache sufferers and no-headache subjects were compared by means of $[chi]^2$ test and computation of the relative risk (RR). Relationships between number of interindividual interactions and PG and pain were analyzed using linear regression, and correlation coefficients are presented to quantify the strength of these relationships. Differences of the differences were compared by means of the t test.

3. Results

3.1. Hypoxia headache sufferers vs nonsufferers

The occurrence of headache at 15 hours after reaching an altitude of 3500m (Fig. 1b) was different in the control (headache=20, no headache=18) and the nocebo group (headache=31, no headache=5) ($[chi]^2$=8.173, P<0.0044; RR=1.63, confidence interval [CI] 1.17–2.27). Mean pain intensity, as assessed by means of a numerical rating scale ranging from 0=no pain to 10=unbearable pain, was 3.9 (CI 3.3–4.4) in the control group and 6.4 (CI 5.9–7) in the nocebo group ([DELTA] means=2.5, CI 1.8–3.3) (Fig. 3). As already observed for blood PG and TX [23], salivary PG and TXA_2 increased from sea level to 3500m in headache sufferers, but not in headache-free subjects, in both the control and the nocebo group (Table 1). However, the increase in the nocebo group was significantly larger for PGE_2, PGF_2, PGI_2, and TXA_2 compared to the control group (Fig. 3). Most interesting, we found a positive correlation between the number of interindividual interactions (encircled numbers in Fig. 1a) and both headache pain intensity [r=0.827, R2=0.685, t(29)=7.942, P<0.0001] and salivary PGE_2 increase [r=0.858, R2=0.736, t(29)=8.994, P<0.0001] (Fig. 4), suggesting an intimate relationship between the amount of negative information received and both pain and PGE_2.

We also measured salivary cortisol and found that, differently from the eicosanoids, whereas cortisol increased at 3500m in the nocebo group, no change occurred in the control group. This suggests that the cortisol increase in the nocebo group was not due to the high altitude per se, but rather, to anxiety induced by the negative expectations ([DELTA] means nocebo vs control=25, 14.3–35.7) (Fig. 3).

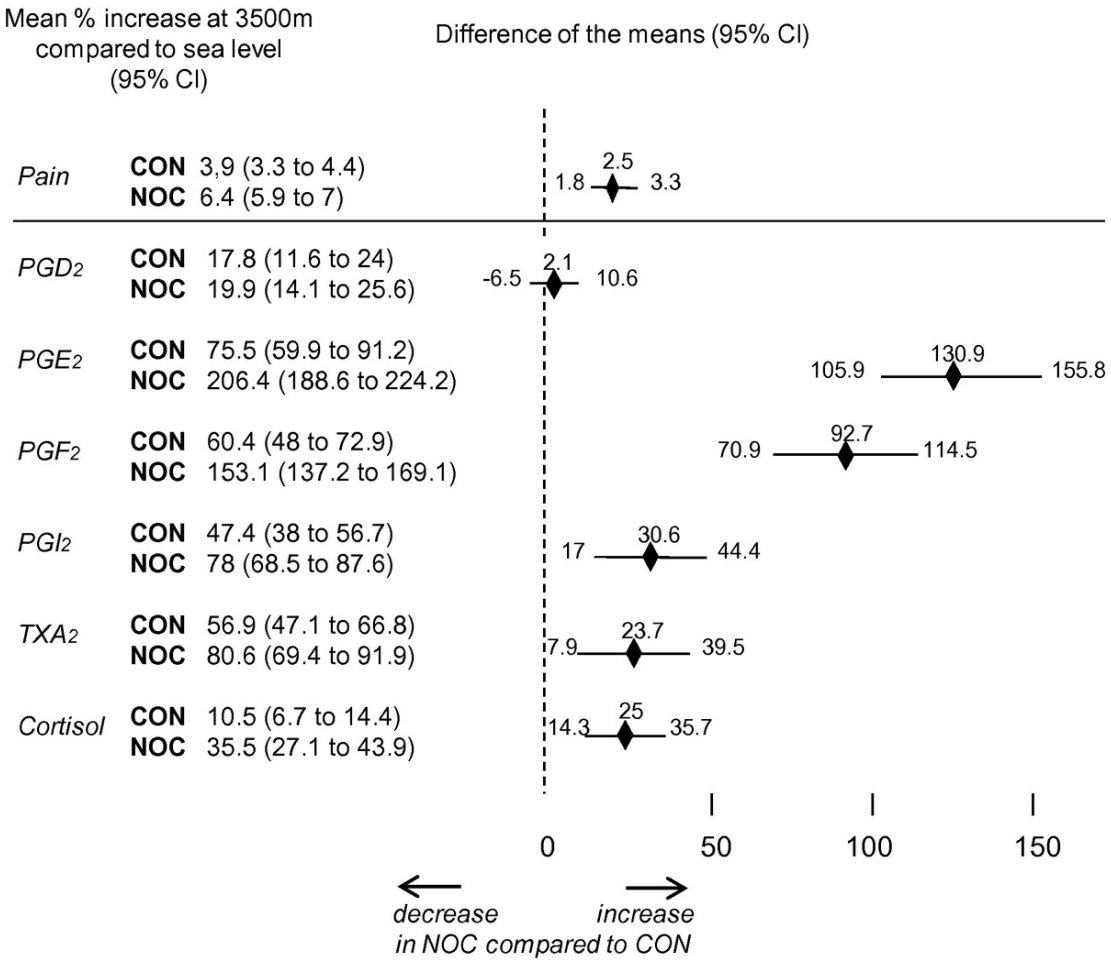

Fig. 3. Increases in headache pain, salivary prostaglandins (PG), thromboxane (TX), and cortisol at 3500 m compared to sea level in both control (CON) and nocebo (NOC) groups. On the left, mean percentage increases and 95% confidence intervals (CI) are shown in both groups. On the right, the differences between the means of the 2 groups are shown with 95% CI. Note that all values are significantly increased in group NOC compared to CON, with the exception of PGD_2.

Table 1. Mean (95% confidence interval [CI]) blood oxygen saturation (SO_2) and salivary concentration of prostaglandins (PG), thromboxane (TX), and cortisol in headache sufferers and headache-free subjects at 3500 m.

		Sea level	3500 m	
Headache sufferers at 3500 m				
Control (n = 20)				
	SO_2[a]	97 (96.5 to 97.5)%[a]	86.2 (85.2 to 87.2)%[a]	$P < 0.02$[a]
	PGD_2	173.8 (144.5 to 203) nmol/mg	205.3 (181.4 to 229.2) nmol/mg	$P = 0.403$
	PGE_2[a]	4.2 (3.1 to 5.3) nmol/mg[a]	7.3 (6.4 to 8.3) nmol/mg[a]	$P < 0.001$[a]
	PGF_2[a]	3.5 (2.6 to 4.3) nmol/mg[a]	5.6 (5 to 6.2) nmol/mg[a]	$P < 0.005$[a]
	PGI_2[a]	15.5 (12.5 to 18.4) nmol/mg[a]	22.8 (18.7 to 26.9) nmol/mg[a]	$P < 0.001$[a]
	TxA_2[a]	28.7 (23.4 to 33.9) nmol/mg[a]	45 (36.9 to 53) nmol/mg[a]	$P < 0.002$[a]
	Cortisol	1.2 (0.8 to 1.5) μg/dL	1.3 (0.9 to 1.6) μg/dL	$P = 0.832$
Nocebo (n = 31)				
	SO_2[a]	97 (96.6 to 97.4)[a]	85 (84 to 86)%[a]	$P < 0.01$[a]
	PGD_2[a]	196 (175.6 to 216.3) nmol/m[a]	235.2 (217.2 to 253.2) nmol/mg[a]	$P < 0.03$[a]
	PGE_2[a]	3.9 (3.1 to 4.7) nmol/m[a]	11.9 (10.2 to 13.6) nmol/m[a]	$P < 0.001$[a]
	PGF_2[a]	4.1 (3.4 to 4.8) nmol/m[a]	10.4 (8.9 to 11.9) nmol/m[a]	$P < 0.002$[a]
	PGI_2[a]	14.1 (11.4 to 16.7) nmol/m[a]	25 (21.5 to 28.5) nmol/m[a]	$P < 0.001$[a]
	TxA_2[a]	35.6 (30.2 to 41) nmol/m[a]	63.6 (58.8 to 73.3) nmol/m[a]	$P < 0.001$[a]
	Cortisol[a]	1.2 (0.9 to 1.5) μg/dL[a]	1.8 (1.6 to 2.1) μg/dL[a]	$P < 0.02$[a]
No headache at 3500 m				
Control (n = 18)				
	SO_2[a]	97.4 (97 to 97.8)%[a]	85.5 (84 to 87)%[a]	$P < 0.03$[a]
	PGD_2	159.8 (137.2 to 182.4) nmol/mg	176.6 (146.5 to 206.7) nmol/mg	$P = 0.546$
	PGE_2	5.2 (3.7 to 6.7) nmol/mg	6.3 (3.9 to 8.7) nmol/mg	$P = 0.63$
	PGF_2	3.7 (2.4 to 4.9) nmol/mg	5.1 (3.2 to 7) nmol/mg	$P = 0.496$
	PGI_2	12.1 (9.7 to 14.5) nmol/mg	17 (13.1 to 20.9) nmol/mg	$P = 0.230$
	TxA_2	24 (19 to 29) nmol/mg	34.4 (27 to 41.7) nmol/mg	$P = 0.333$
	Cortisol	1 (0.7 to 1.4) μg/dL	1.5 (1 to 2.1) μg/dL	$P = 0.384$
Nocebo (n = 5)				
	SO_2[a]	97.2 (95.9 to 98.4)%[a]	86.8 (83.7 to 89.9)%[a]	$P < 0.03$[a]
	PGD_2	180 (112.9 to 247) nmol/mg	195.2 (114.9 to 275.5) nmol/mg	$P = 0.775$
	PGE_2	3.5 (−0.1 to 7.1) nmol/mg	4.9 (1.2 to 8.6) nmol/mg	$P = 0.560$
	PGF_2	4.7 (−0.3 to 9.7) nmol/mg	8 (2.4 to 13.6) nmol/mg	$P = 0.295$
	PGI_2	12.9 (6.7 to 19.1) nmol/mg	15.5 (6.1 to 24.9) nmol/mg	$P = 0.206$
	TxA_2	30.7 (13.3 to 48.1) nmol/mg	33.2 (21 to 45.4) nmol/mg	$P = 0.787$
	Cortisol	1.1 (0.4 to 1.9) μg/dL	1.9 (1.3 to 2.5) μg/dL	$P = 0.654$

Whereas the headache sufferers showed significant changes from sea level to 3500 m, the headache-free subjects showed no changes in salivary PG, TX, and cortisol.
[a] Significant differences (nonoverlapping CI). The *P* values of the comparisons between low and high altitude (*t* test) are also shown.

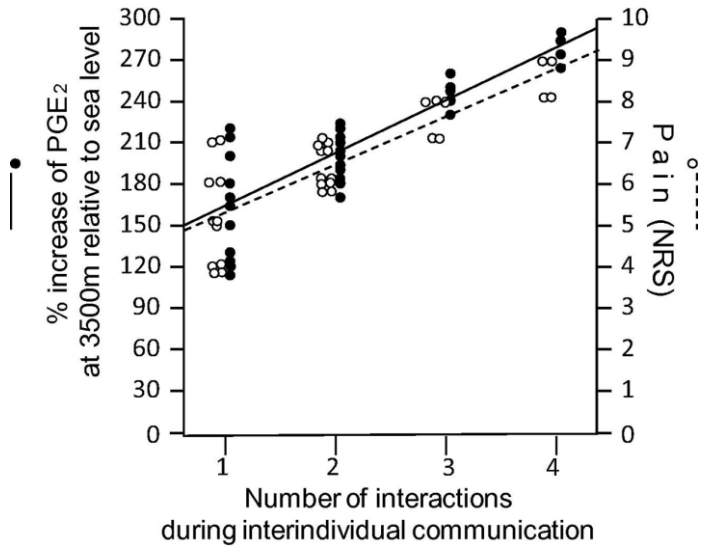

Fig. 4. Correlation between the number of interactions during interindividual negative communication, as represented by the encircled numbers in Fig. 1a, and both salivary prostaglandin (PGE_2) increase (black circles) and headache pain (white circles). NRS, numerical rating scale.

3.2. Aspirin vs placebo vs no treatment in hypoxia headache sufferers

The headache sufferers at 15hours after reaching 3500m were randomly subdivided into 3 groups, and the groups taking either aspirin or placebo were studied according to a double-blind paradigm (Fig. 1b). In the headache sufferers of the control group, we found that a dose of 25mg/kg of aspirin relieved the headache attack and blocked PG and TX increase after 4hours, whereas the placebo group showed a significant effect neither for pain intensity nor for PG-TX (Fig. 5a). In the headache sufferers of the nocebo group we found that a dose of 25mg/kg of aspirin relieved the headache attack and blocked PG and TX increase, as in the control group. By contrast, differently from the control group, the placebo treatment showed a significant effect on both pain and PG-TX (Fig. 5b). The effects of placebo were significantly smaller than those of aspirin, as computed by means of the difference of the differences (ie, comparison of Aspirin-Placebo vs Placebo-No treatment) for pain (-1.70 [CI -2.43 to -0.99], P<0.001), PGE_2 (-59.58 [CI -77.58 to -41.58], P<0.001), PGF_2 (-44.19 [CI -63.56 to -24.83), P<0.001], PGI_2 (-44.19 (CI -63.56 to -24.83], P<0.001), and TXA_2 (-27.74 [CI -36.54 to -18.94], P<0.001).

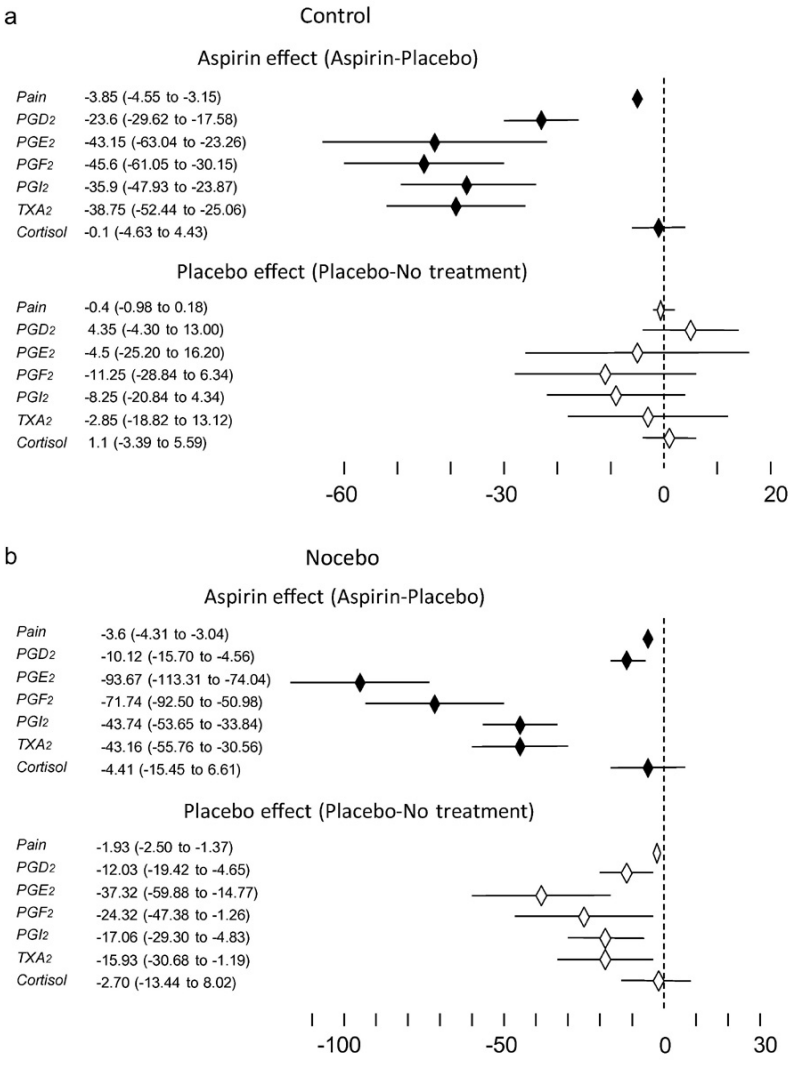

Fig. 5. Aspirin effect and placebo effect in headache sufferers of the control and nocebo groups. (a) Difference between the means of aspirin and placebo (aspirin effect), and between the means of placebo and no treatment (placebo effect) for the control group. (b) Same as in (a), but for the nocebo group. PG, prostaglandins; TX, thromboxane.

The fact that placebo did not have any effect in the control group (Fig. 5a) and that the placebo effect was smaller than the aspirin effect in the nocebo group (Fig. 5b), suggests that placebo acted only on the nocebo-induced increase in PG and TX. This is further supported by the modulation of headache pain by the placebo treatment (Fig. 6). In fact, whereas the no treatment of the nocebo group was more painful than the no treatment of the control group ([DELTA] means=1.9, CI 1.35–2.45), there was no difference between the placebo in the nocebo group and the no treatment of the control group ([DELTA] means=0.0, CI -0.6–0.6), which indicates that placebo in the nocebo group only blocked the nocebo-enhanced component of pain. It is also worth noting that aspirin was effective in both groups, with no difference between them ([DELTA] means=0.6, CI -0.13–1.33), which indicates that the aspirin effect in the nocebo group was the result of both a specific effect of aspirin and a placebo effect.

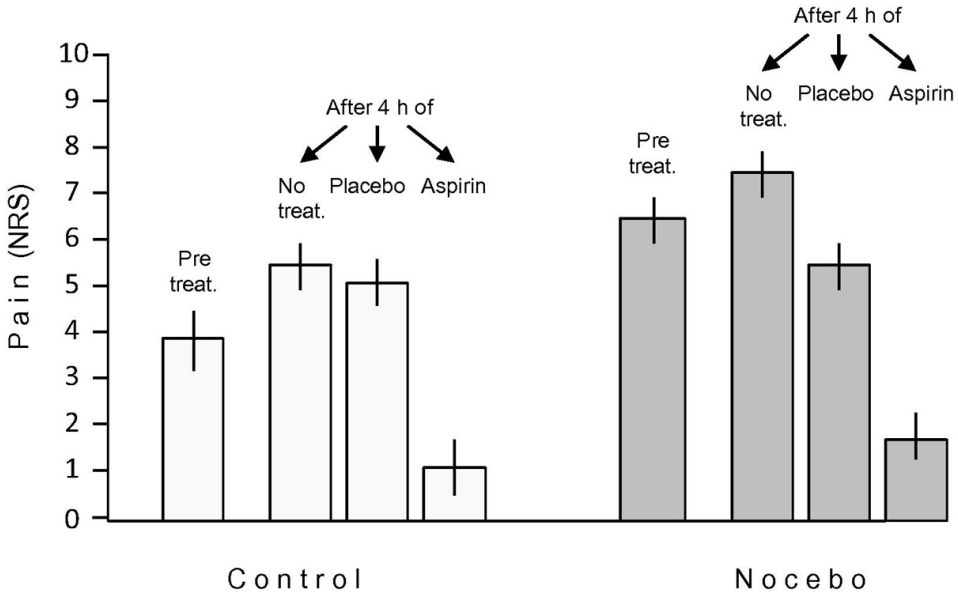

Fig. 6. Headache pain in the control and nocebo group. In the nocebo group, placebo blocked only the nocebo component (difference between no treatment in the control group and no treatment in the nocebo group), whereas it was totally ineffective in the control group. Aspirin was equally effective in the 2 groups. Means and confidence intervals (CI) are shown. Pretreat represents the measurements taken 15 hours after reaching 3500 m. Then, measurements were taken again 4 hours later in the 3 conditions (no treatment, placebo, aspirin). NRS, numerical rating scale.

3.3. Headache sufferers and nonsufferers in nonhypoxic (normoxic) conditions

In order to see if these effects also occurred without the hypoxic stimulation of PG and TX, we repeated the same experiment in an additional nocebo group of 24 subjects who received negative information with the same procedure in a population of 92 subjects. A total of 20 subjects, randomly taken from those who were not reached by the negative information, represented the control group. These nocebo and control groups went up to an altitude of 1500m, where no hypoxia is present (SO_2=96%±1 SD). Headache was reported by 2 of 24 subjects in the nocebo group and by 2 of 20 in the control group ($[chi]^2$=0.112, P=0.737; RR=0.833, CI 0.128–5.396), and the overall salivary concentrations of PGD_2, PGE_2, PGF_2, PGI_2, and TXA_2 were not different from the concentrations at

sea level (Table 2). Interestingly, in this nocebo group we found an increase in salivary cortisol compared to sea level ([DELTA] means=0.5, CI 0.25–0.75), whereas no cortisol increase occurred in the control group, which is in keeping with an increase in negative expectation-induced anxiety. These 2 additional groups show that negative expectations were ineffective if there was no baseline hypoxic stimulation of the COX-PG pathway.

Table 2. Mean (95% confidence interval [CI]) blood oxygen saturation (SO_2) and salivary concentration of prostaglandins (PG), thromboxane (TX), and cortisol in the subjects who went up to 1500 m, thus with no hypoxic stimulation.

	Sea level		1500 m	
Control (n = 20)				
	SO_2	97.6 (97.1 to 98.1)%	97.2 (96.7 to 97.7)%	$P = 0.886$
	PGD_2	168.8 (140.5 to 197.1) nmol/mg	185.3 (160.6 to 210) nmol/mg	$P = 0.704$
	PGE_2	5 (3.6 to 6.4) nmol/mg	4.5 (2.9 to 6.1) nmol/mg	$P = 0.765$
	PGF_2	4 (3.1 to 4.8) nmol/mg	4.6 (4 to 5.2) nmol/mg	$P = 0.655$
	PGI_2	18.3 (14.7 to 21.9) nmol/mg	20.2 (16.4 to 24) nmol/mg	$P = 0.607$
	TxA_2	39 (31.7 to 46.2) nmol/mg	47.8 (42.6 to 52.9) nmol/mg	$P = 0.500$
	Cortisol	1.2 (1 to 1.4) μg/dL	1.1 (0.7 to 1.6) μg/dL	$P = 0.812$
Nocebo (n = 24)				
	SO_2	97.3 (96.9 to 97.7)%	97.9 (97.4 to 98.3)%	$P = 0.694$
	PGD_2	206 (186.7 to 225.3) nmol/mg	184.1 (159.2 to 209) nmol/mg	$P = 0.707$
	PGE_2	3.3 (2.7 to 3.8) nmol/mg	4.3 (3.2 to 5.4) nmol/mg	$P = 0.529$
	PGF_2	6.1 (4.9 to 7.3) nmol/mg	5.5 (4.2 to 6.8) nmol/mg	$P = 0.840$
	PGI_2	19.9 (16.2 to 23.6) nmol/mg	17.6 (13.3 to 21.9) nmol/mg	$P = 0.540$
	TxA_2	40.4 (35.2 to 45.6) nmol/m	36.6 (30.3 to 42.9) nmol/mg	$P = 0.482$
	Cortisol[a]	1.3 (1 to 1.5) μg/dL[a]	1.8 (1.6 to 2) μg/dL[a]	$P < 0.01$[a]

No change from sea level to 1500 m was observed, with the exception of cortisol in the nocebo group.
[a] Significant differences (nonoverlapping CI). The *P* values of the comparisons between low and high altitude (*t* test) are also shown.

4. Discussion

Several important findings emerge from the present study. First, negative expectations in a sample of individuals were capable of changing a specific biochemical pathway related to PG synthesis. Second, negative expectations did not create pain and PG synthesis from nothing, but rather they enhanced the hypoxia-activated COX-PG pathway. Third, it should be noted that a placebo treatment was effective only on the nocebo component of pain/PG increases, whereas it was completely ineffective on the specific hypoxia-related increases. This is an important point, particularly by considering that some people respond to placebos, whereas some other people do not. In our model of hypobaric hypoxia headache, the presence of a nocebo component of pain was fundamental in order for a placebo to work. However, it should be noted that this finding that placebos work to reverse nocebo expectancies cannot be generalized to other conditions. Future studies should clarify this point in other medical conditions, in order to see whether placebos better work on a pain baseline that had been previously increased by a nocebo component.

It is also worth noting that the pathophysiological model of hypobaric hypoxia (or high altitude) headache represents an interesting approach to study the influence of different psychological factors on pain, for at least 2 reasons. First, high altitude headache is a clinical condition that can be

reproduced at will in healthy subjects. Thus, it can be considered to be at the border between the experimental and the clinical setting, with all the advantages of the laboratory on the one hand, and all the advantages of the clinical setting on the other hand. Second, the hypoxia-induced activation of the COX-PG pathway allows us to better identify the changes that are induced in COX activity by different psychological factors, such as placebo and nocebo administration. The lack of any effect at an altitude of 1500m, where no hypoxia is present, supports this view, that is, high altitude as an excellent model to activate PG synthesis. In this regard, it is worth noting that in the present study we were able to show an increase in salivary PG and TX at high altitude, which is in keeping with a similar plasma increase reported in a previous study [27].

Some limitations of this study must be highlighted. First, the present experimental approach cannot unravel why interindividual communication of negative expectations took place. In fact, participants were "self-selected" in the nocebo and control groups, thus, there was no real random assignment. For example, participants high in catastrophic thinking or anxiety might have been more likely to be the communicators or the recipients of pain-related communication, and as such, might be overrepresented in the nocebo group. However, it should be pointed out that our main objective was to induce negative expectations across a sample of individuals, regardless of the baseline characteristics of personality and attitudes. Therefore, from the present study we can conclude that negative expectations can spread across some individuals and can induce biochemical changes, although the underlying mechanism needs to be clarified. Future research should be aimed at assessing whether the same effects occur in a randomized sample of individuals who have been informed about the possible occurrence of headache by the experimenters themselves, without using the interindividual communication model of the present study.

A second limitation is that we studied a specific condition—hypobaric hypoxia headache—thus, these effects are not necessarily present in other medical conditions, where other mechanisms might be involved. Nonetheless, it is important to point out that we used hypoxia in order to activate the COX-PG pathway, thus, other types of COX-PG activation, such as inflammation, might produce similar effects.

Although from the present study it is not possible to know the mechanisms through which negative expectations led to the increase in PG synthesis, it is interesting to note that negative expectations also induced an increase in cortisol, and glucocorticoids have been found to have a facilitating effect on the COX pathway in some circumstances, such as pregnancy and parturition [10,36,37]. Therefore, it is tempting to speculate that the nocebo-induced cortisol increase in our hypoxic condition may have enhanced PG synthesis, although it should be noted that we found no correlation between cortisol and PG increases.

The mere observation of others has been shown to induce both placebo [14] and nocebo effects [30,33], thus indicating that positive and negative expectations can be triggered by social interactions and communication. In addition, Colloca and Benedetti [14] showed that the magnitude of placebo analgesia following the observation of others can be as large as placebo analgesia evoked by a previous first-person conditioning procedure. This underscores the important role of social interaction and communication in the modulation of pain, as previously shown in a number of circumstances (eg, [28]).

It is interesting to note that, in our experimental condition, negative information propagated across 36 subjects in 1week, thus, a hundred/thousand subjects might get involved in longer periods of times. This could represent an interesting model for future research to analyze the propagation of negative information across a large sample of individuals. The study of social networks over the past years has shown the importance of social interaction and communication in both human behavior [11,17] and

health [22,29]. Interindividual propagation of behaviors and attitudes is common in a variety of situations, such as the social propagation of emotions [9,18], smoking cessation [12], obesity [13], and suicide [1]. Overall, these studies suggest that the social environment can be an important contributor to health, and emphasize how negative expectations can propagate across a large number of individuals, thus contributing to the dissemination of symptoms and illness across the general population.

Our results emphasize once again the notion that nocebos and placebos may act on the same biochemical pathways that are used by drugs, as previously shown in a number of studies (see [2] for a recent review). The COX-PG pathway is an important target of many analgesic drugs, thus, its involvement in nocebo hyperalgesia and placebo analgesia may represent an important confounding factor in the clinical trials setting.

Conflict of interest statement

The authors declare no competing financial interests.

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