Effect of 15(R)-15-methyl-prostaglandin E_2 on iron absorption in the rat[1]

A. Gutnisky, P. Glikman, M.F. Gimeno and A.L. Gimeno[2]

Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) de la República Argentina, Serrano 665/69, 1414 Buenos Aires (Argentina), 1 June 1981

Summary. The administration of 15(R)-15-methyl prostaglandin E_2 (15(R)-15-M-PGE_2) in vivo significantly diminished the uptake of ^{59}Fe into blood, spleen, liver, femur and dried intestine of rats, whereas acetylsalicylic acid (ASA) increased the counts significantly. This effect of ASA was counteracted by 15(R)-15-M-PGE_2. It is suggested that prostaglandins (PGs) might play an important role in inhibiting iron absorption at the intestinal level.

One of the aspects of iron metabolism, the intestinal absorption, is the initial step of a complex process which is followed by its transport, storage, utilization and elimination[3]. The intestinal epithelium has, under normal conditions, a transference system able to regulate iron absorption according to requirements[4]. The transport mechanism for iron at the intestinal level is apparently saturated (under certain conditions) either at the level of incorporation into the intestinal cells or of the transference from them into the circulation[5].

The experimental findings are, however, contradictory, depending on the methods employed in different studies, i.e. variable results have been obtained in vivo or in vitro, and also with the employment of 'intestinal loops' in animals or tests in human beings[6-8]. We have tried to clarify, using in vitro experiments, the role of erythropoietin on iron transport across the isolated intestinal tract of the rat[9]. The participation of the prostaglandins (PGs) E_1 and E_2 in the complex process of intestinal iron absorption has also been previously explored in vivo and in vitro and so has that of acetylsalicylic acid (ASA)[10,11]. It was found that both PGE_1 and PGE_2 can influence iron uptake by the villous cells of the intestine in different ways, and they also influence the processes by which iron is transported across the serosal pole into the circulation. The finding that ASA favoured iron absorption in vivo[11] and the knowledge that this agent is a recognized inhibitor of the synthesis of PGs, blocking the cyclooxygenase system[12] prompted us to perform the present expriments aimed at exploring the influences of the oral administration of 15(R)-15-methyl-prostaglandin E_2 (15(R)-15-M-PGE_2) on iron absorption in vivo. The effects of ASA were also tested simultaneously.

Methods. Male Wistar rats (200±20 g) were bled by cardiac puncture 1.5 ml/100 g b.wt during 2 consecutive days. 24 h later another bleeding of 0.75 ml/100 g b.wt was performed. After these 3 days of bleeding the animals developed an anemia with hemoglobin values below 10 g%. The rats were then starved for 24 h and forthwith divided into 4 groups: group I (control), received 0.5 ml of NaCl 0.9% via a catheter placed in the stomach and was given ^{59}Fe (1 μCi equivalent to 500,000–600,000 cpm) 30 min later, together with an iron carrier, ferrous sulphate (2×10^{-4} M); group II was treated in the same way as group I but also received 10 mg of ASA; group III was treated with 1 μg of 15(R)-15-M-PGE_2 (kindly provided by Dr J. Pike, The Upjohn Co., Kalamazoo, Michigan) dissolved in 0.5 ml of saline, and given ^{59}Fe 30 min later; group IV was treated like the preceding one but also exposed to ASA (10 mg) jointly with ^{59}Fe.

The amount of ^{59}Fe was afterwards monitored in blood, liver, spleen, femur and intestine, the determinations being made 180 min after the delivery of ^{59}Fe. In the case of intestine the ^{59}Fe uptake was determined immediately after removal, as well as after washing the lumen with 100 ml of saline or after desiccation during 24 h at 80 °C. The dry and wet weights of the intestine and the wet weights of spleen and liver were also obtained and the results calculated as cpm of ^{59}Fe per 100 mg of organ. Counts in blood and femur were expressed per 100 g b.wt and per femur, respectively. For statistical assessment the results were compared employing Student's t-test and differences between means were considered significant if $p=0.05$ or less.

Results. The table summarizes the results obtained. No significant differences were detected in body weight, hemoglobin or hematocrit, suggesting that the 4 groups studied were in comparable condition. The pretreatment with

Effects of 15(R)-15-methyl prostaglandin E_2 (15(R)-15M-PGE_2) and acetylsalicylic acid (ASA) on the incorporation of orally given ^{59}Fe into several tissues*

	Group I Controls (9)	Group II ASA (9)	Group III 15(R)-15M-PGE_2 (9)	Group IV 15(R)-15M-PGE_2 + ASA (9)
Body weight (g)	183±3.0	190±6.0	191±4.0	196±2.0
Hemoglobin (g%)	9.1±0.1	8.8±0.2	8.9±0.1	8.6±0.3
Hematocrit (%)	32±0.4	30±1.0	31±0.5	30±1.0
Blood (cpm in 2 ml/100 g b.wt) 10^{-2}	83.5±7.6	148±6.5	46.1±10.7	91.0±21.8
Spleen (cpm/100 mg w.wt) 10^2	31.6±4.2	51.1±13	15.4±3.5	31.3±7.1
Liver (cpm/100 mg w.wt) 10^3	15.8±1.5	55.0±8.0	10.4±1.7	16.5±3.4
Wet washed empty intestine (cpm/100 mg w.wt) 10^{-2}	10.8±1.4	8.0±1.4	9.8±1.5	10.0±1.2
Freshly removed intestine (cpm/100 mg w.wt) 10^{-2}	68.3±9.9	61.1±11.7	76.1±15.8	52.9±12.1
Dry intestine (cpm/100 mg w.wt) 10^{-2}	69.6±5.2	67.0±7.0	38.3±6.0	36.4±6.0
Femur 10^2	48.6±6.8	98.4±17.0	23.5±5.6	45.3±12.1

* Determinations made at 180 min following ingestion (see 'Methods' section). Means±SEM. Figures between parentheses indicate the number of cases.

15(R)-15-M-PGE_2 (group III) prior to the administration of ^{59}Fe was accompanied by a significant reduction of counts in comparison with controls (group I) in blood ($p < 0.02$) spleen ($p < 0.05$), liver ($p < 0.02$), femur ($p < 0.01$) and dried intestine ($p < 0.02$). On the other hand, following the administration of ^{59}Fe accompanied by ASA (group II) there is a significant increase of counts in comparison with controls (group I) in blood ($p < 0.02$), spleen ($p < 0.02$), liver ($p < 0.002$) and femur ($p < 0.005$). No differences were observed in dried intestine.

Results obtained with ASA (groups II) were reversed to control values (group I) when the animals were pretreated with 15(R)-15-M-PGE_2 (group IV) in blood, spleen, liver and femur whereas in dry intestine counts were significantly lower ($p < 0.002$). In freshly removed as well as in wet-washed empty intestine counts were not significantly different in the 4 groups.

Discussion. The foregoing results document that in the rat the action of ASA is to augment the absorption of iron. This effect is clearly observed at 180 min following the administration of ^{59}Fe via a catheter placed in the stomach. Indeed, an enhanced count of ^{59}Fe was found in blood, spleen, liver and femur and comparable results were detected in the intestine. These results are in keeping with previous findings[11] suggesting that the enhanced intestinal absorption is followed by higher circulating iron levels and enhanced deposit in storage organs.

The administration of a methyllated derivative of PGE_2 diminished iron absorption, circulation and deposit in storage organs. These findings support our previous observations in vitro regarding the effect of PGE_1 and PGE_2 on iron transport across the isolated intestine of the rat. Therefore, it is documented for the first time that among the several actions on the intestinal tract ascribed to PGs there is a distinct influence inhibiting iron absorption both in vivo and in vitro.

On the other hand, the 15(R)-15-M-PGE_2 was able to reverse the stimulatory action of ASA on iron absorption. This suggest that the increment of iron absorption produced by ASA is related to an inhibition of prostaglandin synthesis via the blockade of the intestinal cyclooxygenase system. The findings suggest that endogenous PGs may play an important role influencing iron absorption at the intestinal level.

1 This work was supported by grant No. 6638 from CONICET (Argentina). The technical assistance of Mrs María E. Castro and Norma Rizzo is gratefully acknowleged.
2 Reprint requests should be addressed to: A.L. Gimeno, CEFAPRIN, Serrano 665/69, 1414 Buenos Aires (Argentina).
3 E.L.W. Powell, L.W. Halliday and M.L. Barret, Aust. N.Z.J. Med. *9*, 578 (1979).
4 G. Becker, S. Korpilla-Schäfer, K. Osterloh and W. Forth, Blut *38*, 127 (1979).
5 M.S. Wheby, L.G. Jones and W.H. Crosby, J. clin. Invest. *43*, 1433 (1964).
6 D. Gitlin and A. Cruchaud, J. clin. Invest. *41*, 344 (1962).
7 P.F. Hahw, E.L. Carothers, W.J. Darly, M. Marin, C.W. Sheppard, R.O. Beam, P.M. Densen, J.C. Petterson and C.S. McClellan, Am. J. Obstet. Gynec. *61*, 477 (1951).
8 H.C. Heinrich, in: Intestinal Iron Absorption, Methods of Measurement, Dose Relationship, Diagnostic and Therapeutic Application, Iron Deficiency p. 213. Academic Press, London/New York 1970.
9 A. Gutnisky, E. Speziale, M.F. Gimeno and A.L. Gimeno, Experientia *35*, 623 (1979).
10 A. Gutnisky, E. Speziale, M.F. Gimeno and A.L. Gimeno, Acta physiol. latinoam. *30*, 217 (1980).
11 P. Glikman, A. Gutnisky, M.F. Gimeno and A.L. Gimeno, Experientia *37*, 589 (1981).
12 R.J. Flower, Pharmac. Rev. *26*, 33 (1974).

Effects of L-aspartic acid, L-asparagine and/or L-asparaginase on forced swimming-induced immobility, analgesia, and decrease in rectal temperature in rats

H. Koyuncuoğlu, L. Eroğlu and T. Altuğ

Max-Planck-Institut für experimentelle Medizin, D-3400 Göttingen (Federal Republic of Germany), and Department of Pharmacology and Clinical Pharmacology, Istanbul Medical Faculty, Istanbul (Turkey), 14 April 1981

Summary. The effect of L-aspartic acid, L-asparagine and/or L-asparaginase were compared with those of imipramine on immobility, number of defecations, increase of nociceptive threshold, and hypothermia, induced by forced swimming in rats. L-Aspartic acid was found to be as effective as imipramine in reducing the effects of forced swimming, presumable by normalizing the decreased level of endogenous L-aspartic acid, due to the inhibition of L-asparaginase activity and/or by stimulating the inhibited enzyme. The other treatments antagonized the immobility, but not the increased number of defecations. All compounds abolished the elevation of nociceptive threshold and hypothermia.

There are few, if any, animal models which both resemble the clinical phenomenon of depressive illness and are selectively sensitive to treatments known to be effective in its management. Porsolt et al.[1,2] have recently proposed that the immobile behaviour observed during forced swimming in rats and mice may serve as a screening model for potential antidepressants, and have shown that it was reduced by a variety of drugs that are therapeutically effective in depression. As a stressful event, forced swimming also produces analgesia in rats[3–5]. Akil et al.[6] and Chance et al.[7] have attributed the analgesia induced by stressful events to endogenous opioids whereas other workers[3–5] claim that there is little, if any, relationship between this analgesia and endorphin system in the brain. In addition to the many well-known similar effects of endogenous opioids and opiates they both affect central thermoregulation[8] and beta-endorphin administered into the lateral ventricle of rats causes, like opiates, a profound state of immobilization characterized by the absence of spontaneous movements. On the other hand, morphine has been shown to cause increases and decreases in the activity of L-asparaginase[9] which can be antagonized by L-aspartic acid. Additionally L-aspartic acid antagonizes the acute and chronic effects of morphine[9,10] and the manipulation of L-asparaginase activity by administering D- and/or L-aspartic acid causes body weight loss, decreases in food and fluid intakes[11], naloxone reversible decrease in rectal temperature, increases in the releases of ACTH, vasopressin and