Differential regional changes of prostacyclin and thromboxane A$_2$ synthesis in the intestinal tract of the fasted and semistarved rat

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Abstract. The synthesis of prostacyclin (PGI$_2$) and thromboxane A$_2$ (TXA$_2$) by the mucosal and muscular portions of the duodenum, jejenum, ileum and ascending colon, as well as that by mesenteric vessels, was investigated in starved and semistarved rats. The jejunal mucosa and muscularis showed a marked increase in PGI$_2$ synthesis after fasting for 48 h and 72 h or semistarvation for 9 days compared with controls. Jejunal TXA$_2$ synthesis did not alter. In contrast, PGI$_2$ and TXA$_2$ synthesis in ileal mucosa and muscularis was significantly reduced after fasting for 48 h, 72 h and semistarvation for 9 days. PGI$_2$ and TXA$_2$ synthesis by duodenal and colonic muscularis was unaffected by fasting or semistarvation. PGI$_2$ synthesis in mesenteric vessels was significantly increased by fasting and semistarvation. No changes in PGI$_2$ or TXA$_2$ were detected at 24 h in fasted rats in any of the tissues studied when compared with controls. These selective changes in PGI$_2$/TXA$_2$ secretion may be important mediators of adaptive changes in the small intestine in response to starvation.

Key words: Prostacyclin — Thromboxane A$_2$ — Small intestine — Mesenteric vasculature — Fasting — Semistarvation

Introduction

Mesenteric vascular flow (Mailman 1982; Winne 1979) and the motility of the gut (Sarr et al. 1980; Holgate and Read 1983) are two important regulators of nutrient absorption from the gut. Since prostacyclin (PGI$_2$) and thromboxane A$_2$ (TXA$_2$) affect mesenteric blood flow (Dusting et al. 1978; Soma et al. 1985; Traverso et al. 1984; Kauffman et al. 1982; Whittle et al. 1981) and gut motility (Nakahata and Suzuki 1981; Coleman et al. 1981; Gaion and Trento 1983), they may play a role in the regulation of absorption of nutrients from the small intestine. Total starvation and semistarvation are known to induce profound changes in intestinal structure and function: reduction in mucosal mass (Steiner et al. 1968), regional changes in motility (Sanford and Smyth 1975; Poulakos and Kent 1973) and alterations in nutrient uptake (Debnam and Levin 1975; Kotler et al. 1981; Debnam and Thompson 1985; Hindmarsh et al. 1967). In view of the above, we undertook an investigation of PGI$_2$ and TXA$_2$ synthesis by the mesenteric vessels and muscularis layers of the duodenum, jejunum, ileum and colon and the mucosal layers of the jejunum and ileum.

Materials and methods

All experiments were carried out using male Sprague Dawley rats of initial body weight 250—300 g. Rats in the fed group were maintained on Diet 41 B (Grain Harvester, Kent, UK) and allowed free access to water. Fasted animals had their food removed for 24 h, 48 h or 72 h prior to experimentation. The semistarved rats were each given 9 g of food each day for 9 days. All diet-restricted rats were allowed unlimited access to water and caged individually in wire-bottomed cages to minimise coprophagy. Weight changes were monitored in all experimental groups (see Results).

Rats were anaesthetised with pentobarbitone (90 mg · kg$^{-1}$ intraperitoneally; Sagatal: May and Baker Ltd., Dagenham, UK). A midline incision was made into the abdominal cavity and sections of duodenum, jejenum, ileum, colon and mesenteric vessels were rapidly dissected out and placed in Dulbecco's minimum essential medium (MEM; containing 12.5 mmol · l$^{-1}$ NaHCO$_3$, gassed with O$_2$/CO$_2$, 95/5; Gibco Biocult, Paisley, Scotland). MEM was used since this media is used routinely in our laboratory for studies on prostanooid synthesis by mammalian tissues (Jeremy et al. 1985a, 1986a, b). The intestinal segments were opened by longitudinal incision and rinsed free of residual food particles. Mucosa was separated from the muscular portion of the tissue with a glass slide (Debnam and Levin 1975) and placed in MEM. The muscular portion was cut into a 2 mm wide strip with a scalpel blade and then into 2 mm squares. Ten squares, in duplicate for each animal, were placed in 1 ml MEM in polypropylene tubes, the tubes stoppered and incubated in a shaking water bath at 37°C for 30 min. Following incubation, the tubes were centrifuged at 2000 rpm for 10 min and an aliquot of supernatant taken and stored at —70°C prior to the measurement of 6-oxo-PGF$_1$α and thromboxane B$_2$ (the stable, spontaneous hydrolysis products of PGI$_2$ and TXA$_2$ by specific radioimmunoassay. Tissue from each tube was washed quickly with distilled water, placed in a desiccator until dry, and weighed. Mucosa was washed three times in MEM, followed by centrifugation at 2000 rpm. The mucosal scrape from each intestinal portion was placed in 1 ml MEM, and incubated and processed as for the muscular tissue, for assessment of PGI$_2$ and TXA$_2$ synthesis.

Mesenteric vessels were dissected away from adherent adipose tissue and cut into 1 mm lengths of vessel for each

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animal, and 10 segments placed in 1 ml MEM, incubated and processed for PGI\textsubscript{2} release as described above. TXA\textsubscript{2} synthesis was not investigated, since we were not able to detect release of this eicosanoid by mesenteric vessels in pilot experiments.

Radioimmunoassay of TXB\textsubscript{2} and 6-oxo-PGF\textsubscript{1α}: validation of methods. Antisera of high serological specificity were purchased from Capell Laboratories (West Chester, PA., USA); \textsuperscript{3}H-6-oxo-PGF\textsubscript{1α} (120 Ci·mmol\textsuperscript{-1}) and \textsuperscript{3}H-TXB\textsubscript{2} (110 Ci·mmol\textsuperscript{-1}) were purchased from New England Nuclear (NEN; Dreieich, FRG) and unlabelled ligand from Sigma Chemical Company (Poole, Dorst, UK). Assay procedures (buffers, equilibration times, separation of bound and unbound ligands, etc.) were carried out according to protocols obtained from Capell Laboratories. Aliquots of incubation supernatant were diluted in assay buffer, and a further aliquot taken for assay. The criteria of validity were derived from definitions stated in the British Medical Bulletin (Sonksen 1974). Lower limit of detection was established as 15 pg for 6-oxo-PGF\textsubscript{1α} and 10 pg for TXB\textsubscript{2}. The intraassay coefficient of variation for 7 separate samples of jejunum (muscularis + mucosa) was 6% and 7% for 6-oxo-PGF\textsubscript{1α} and 4% and 6% for TXB\textsubscript{2}. The interassay coefficient of variation from 10 consecutive assays of a single incubate was 8% for 6-oxo-PGF\textsubscript{1α} and 11% for TXB\textsubscript{2}. Incubation of tissue with indomethacin (Jeremy et al. 1985a) abolished detectable quantities of prostanoid from supernatants. Possible loss of TXB\textsubscript{2} and 6-oxo-PGF\textsubscript{1α} (due to metabolism or reuptake by tissue) was studied by incubating the tissues with \textsuperscript{3}H-TXB\textsubscript{2} and 6-oxo-PGF\textsubscript{1α} for 1 h. The supernatants were acidified to pH 4 and extracted with ethyl acetate. The extract was evaporated and run on thin layer chromatography plates and profiles of radioactive distribution compiled (Jeremy et al. 1985b), revealing no further metabolism of the ligands to more or less polar compounds. Recovery of labelled ligand was 100%, indicating no reuptake of the prostanoids by the tissue. Studies on indomethacin-treated tissues with varying known amounts of unlabelled 6-oxo-PGF\textsubscript{1α} and TXB\textsubscript{2} added were accurately measurable by radioimmunoassay following a 1 h incubation. These studies confirmed the stability of these prostanoids in this system.

Statistics. Results are expressed as pg eicosanoid · mg\textsuperscript{-1} tissue (dry wt.) liberated in 1 min (median; range in parentheses). Animal weights were also expressed as median and range. Mann-Whitney test (two-tailed) was applied for statistical analysis.

Results

Dietary restriction of 24 h, 48 h or 72 h duration caused a progressive decrease in body weight (grams), from an initial weight (expressed as median and range) of 261 (250–275) to 239 (224–248), 223 (210–238), 209 (196–224) (n = 7 for each group) respectively. The weight loss represented 8.7%, 14.6% and 20% of the initial body wt., p < 0.001, in all cases.

Fed animals gained weight over a 72 h period. Their weights went up from 247 (224–257) to 269 (241–284) (n = 7). This represented an increase of 8.7% (p < 0.001).

Animals semistarved, like the other dietary restricted groups, lost weight. Their weights went from 248 (229–277) to 203 (187–239) (n = 7), a loss of 17.8% (p < 0.001), whereas the weights of control animals went from 265 (233–277) to 320 (295–341) (n = 6), an increase of 24% (p < 0.001).

i. Mesenteric vessels

No difference in PGI\textsubscript{2} output was detected between fasted or control mesenteric vessels at 24 h. PGI\textsubscript{2} output from mesenteric vessels was significantly enhanced following a fast of 48 h or 72 h, or 9 days semistarvation when compared with controls (Table 1.1). Since mesenteric vessels did not synthesise detectable quantities of TXA\textsubscript{2}, only PGI\textsubscript{2} was measured in this tissue.

ii. Jejunum

No difference in PGI\textsubscript{2} or in TXA\textsubscript{2} output was seen following a 24 h fast. However, a 48 h or 72 h fast or 9 days semistarvation caused a significant increase in PGI\textsubscript{2} output by the fasted jejunal mucosa (Table 1.3) and underlying muscle layer when compared with controls (Table 1.2). In contrast, there were no significant changes in TXA\textsubscript{2} output following a fast of 24 h, 48 h or 72 h or 9 days semistarvation in the jejunal mucosa (Table 1.3) or underlying muscle layer (Table 1.2).

iii. Ileum

In contrast to jejunal PGI\textsubscript{2} synthesis, PGI\textsubscript{2} output was significantly diminished in ileal mucosa (Table 1.4) and muscularis of fasted animals when compared with controls (Table 1.5). Interestingly, TXA\textsubscript{2} output was similarly reduced in the ileal mucosa (Table 1.5) and muscular portion when compared with controls (Table 1.4).

iv. Duodenum

No significant differences in PGI\textsubscript{2} or TXA\textsubscript{2} output were detected from the duodenal muscular portion of the fasted or 9 day semistarved rat (Table 1.6). Duodenal mucosa was not investigated, since in pilot experiments on day 3 fasted and 9 day semistarved rats no changes in duodenal mucosal PGI\textsubscript{2} or TXA\textsubscript{2} were detected.

v. Colon

There were no significant change in PGI\textsubscript{2} or TXA\textsubscript{2} production by the muscularis of the ascending colon following fasting or semistarvation (Table 1.7). Colonic mucosa was not investigated, since the colon possesses a relatively small mucosa, and no overall changes in eicosanoid synthesis were detected in the colonic mucosa in pilot experiments on day 3 fasted or 9 day semistarved rats.

Discussion

In the present study, marked differential changes in PGI\textsubscript{2} and TXA\textsubscript{2} synthesis were detected in the mucosa and muscularis of the jejunum and the ileum and in the mesenteric vasculature, whereas no changes were detected in the duodenum and ascending colon. These data may be relevant to nutrient absorption, since 1) PGI\textsubscript{2} and TXA\textsubscript{2}